2016-2709

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

ILLUMINA, INC., and ILLUMINA CAMBRIDGE LTD.,

Plaintiffs-Appellees

v.

QIAGEN, N.V., QIAGEN GmbH, QIAGEN GAITHERSBURG, INC., QIAGEN SCIENCES, LLC, QIAGEN INC. (USA), QIAGEN REDWOOD CITY, INC., and INTELLIGENT BIO-SYSTEMS, INC.,

Defendants-Appellants.

Appeal from the United States District Court for the Northern District of California in Case No. 3:16-cv-02788, Judge William H. Alsup

CORRECTED BRIEF OF DEFENDANTS-APPELLANTS

[NON-CONFIDENTIAL VERSION]

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CERTIFICATE OF INTEREST

1. Full Name of Party Represented by me	2. Name of Real Party in interest represented by me is:	3. Parent corporations and publicly held companies that own 10% or more of stock in party
QIAGEN, N.V.	QIAGEN, N.V.	None
QIAGEN GmbH	QIAGEN GmbH	QIAGEN Deutschland Holding GmbH; QIAGEN, N.V.
QIAGEN Gaithersburg, Inc.	QIAGEN Gaithersburg, Inc.	QIAGEN North American Holdings, Inc.; QIAGEN US Finance Holdings (Luxembourg) SARL; QIAGEN, N.V.
QIAGEN Sciences, LLC	QIAGEN Sciences, LLC	QIAGEN Gaithersburg, Inc.; QIAGEN North American Holdings, Inc.; QIAGEN US Finance Holdings (Luxembourg) SARL; QIAGEN, N.V.
QIAGEN Inc. (USA)	QIAGEN Inc. (USA)	QIAGEN Gaithersburg, Inc.; QIAGEN North American Holdings, Inc.; QIAGEN US Finance Holdings (Luxembourg) SARL; QIAGEN, N.V.
QIAGEN Redwood City, Inc.	QIAGEN Redwood City, Inc.	QIAGEN North American Holdings, Inc.; QIAGEN US Finance Holdings (Luxembourg) SARL; QIAGEN, N.V.
Intelligent Bio-Systems, Inc.	Intelligent Bio-Systems, Inc.	QIAGEN North American Holdings, Inc.; QIAGEN US Finance Holdings (Luxembourg) SARL; QIAGEN, N.V.

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or are expected to appear in this court (and who have not or will not enter an appearance in this case) are:

Farella Braun & Martel LLP: Winston Liaw.

Dated: October 14, 2016

By: /s/ *Robert R. Baron, Jr.*

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CONFIDENTIAL MATERIAL OMITTED

The material omitted on page 6 describes QIAGEN and Illumina market share. The material omitted on page 19 describes QIAGEN and Illumina market size and share. The material omitted on page 20 describes Illumina market share. The material omitted on page 21 describes Illumina market share, the effect of GeneReader on Illumina placements, and QIAGEN's investment in GeneReader's development. The material omitted on page 22 describes QIAGEN sales and sales forecasts. The material omitted on page 56 describes Illumina market share. The material omitted on page 58 describes QIAGEN sales. The material omitted on page 61 describes QIAGEN's market share.

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STATEMENT OF RELATED CASES

The Trustees of Columbia Univ. in the City of N.Y. v.Illumina, Inc., No. 12-cv-0376 (D. Del.) (Sleet, J.).

JURISDICTIONAL STATEMENT

On September 9, 2016, the United States District Court for the Northern District of California (Alsup, J.) issued an Order Granting Motion for Preliminary Injunction against QIAGEN (the "Preliminary Injunction Order"). Appx1. After the lower court's September 22, 2016 ruling granting in part Defendants-Appellants' motion to modify and to stay the Preliminary Injunction Order, on September 23, 2016, Defendants-Appellants noticed this appeal as to the lower court's decision granting a preliminary injunction. Appx93. The Court has jurisdiction over this interlocutory appeal under 28 U.S.C. § 1292(c)(1) and § 1295(a)(1).

PRELIMINARY STATEMENT

The lower court recognized that a preliminary injunction is a "rare" remedy, and that this is a "close" case. Appx16; Appx2133. Nevertheless, the court granted this extraordinary remedy and barred QIAGEN from further sales of its "GeneReader" DNA sequencer based on Illumina's allegations of infringement of its United States Patent No. 7,566,537 (the "'537 Patent").[1] The lower court's

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[1] "QIAGEN" refers collectively to Defendants-Appellants QIAGEN, N.V., QIAGEN GmbH, QIAGEN Gaithersburg, Inc., QIAGEN Sciences, LLC, QIAGEN Inc. (USA), QIAGEN Redwood City, Inc., and Intelligent Bio-Systems, Inc. ("IBS"). "Illumina" refers collectively to Plaintiffs-Appellees Illumina, Inc. and Illumina Cambridge Ltd.

order was an abuse of discretion because its rulings that QIAGEN was unlikely to prevail in showing that the '537 Patent is invalid, and that Illumina showed a substantial threat of irreparable harm absent an injunction, were based on errors of law and clearly erroneous factual findings. Accordingly, this Court should reverse the grant of a preliminary injunction, and remand the case for further proceedings.

The claims of the '537 Patent are directed to a method of labeling nucleic acid molecules using modified "nucleotides" or "nucleosides." Nucleotides are the building blocks of DNA and, when linked together, they form a chain of DNA that contains the genetic information of living organisms. Each nucleotide is made up of a sugar molecule, one or more phosphate groups, and a base—either "A," "T," "G," or "C." The order of bases determines the sequence or genetic code in the DNA chain, and the chain is formed by bonds between the nucleotides' phosphate groups and their "hydroxyl group" ("OH") at the "three prime" (or "3′") position of their sugars.

The '537 Patent discloses methods of labeling using nucleotides or nucleosides that have been modified to have: (1) a detectable label attached to the base of a nucleotide or nucleoside by a cleavable link, with the label identifying the base as A, T, G, or C; and (2) a cleavable "protecting group" attached to the sugar molecule at the 2′ or 3′ position that prevents the natural linking process between nucleic acids. One use for these modified, labeled nucleic acids is generally

known as "sequencing-by-synthesis," or "SBS." By incorporating modified nucleotides one-by-one, researchers are able to determine the order of DNA bases, and thus the sequence of the genetic information contained in the DNA. Once a base is read, the protecting group and label can be removed ("cleaved") and the next labeled nucleotide can be added to the growing chain.

In opposing Illumina's motion for a preliminary injunction, QIAGEN presented unrebutted expert evidence that the '537 Patent is invalid for failing to enable all of its claims, and as obvious over the prior art. Illumina did not offer any expert testimony regarding the validity of the '537 Patent. QIAGEN also presented unrebutted evidence acquired from Illumina during an expedited discovery process that Illumina did not suffer any lost sales or price erosion as to its own DNA sequencing instruments in the over eight months GeneReader had been on the market. Even so, the lower court granted the injunction, and held both that QIAGEN had not shown a "substantial question" as to the validity of the '537 Patent, and that Illumina had demonstrated a threat of irreparable harm should the GeneReader be permitted to remain on the market pending the trial scheduled for November 2017. The court's ruling was wrong as a matter of law and of the facts.

First, the lower court made an error of law by construing the "nucleoside" limitation in the '537 Patent as including nucleosides combined with phosphate groups, contradicting the definition of nucleoside in the patent.

Specifically, Illumina defined the term nucleoside in the '537 Patent as "missing phosphate [groups]," but the court, without support in the '537 Patent's specification or claims, held that a "skilled artisan would have understood the reference to nucleosides in the patent claims to include the nucleosides that, when ultimately combined with a phosphate group, become nucleotides." Appx14. The lower court's claim construction error was determinative as to QIAGEN's enablement argument; when correctly defined in accordance with the plain terms of the '537 Patent, a nucleoside ***cannot be incorporated*** as the method requires, and therefore the patent's claims are not enabled.

Second, the lower court misapplied the law in concluding that QIAGEN failed to raise a substantial question as to enablement with regard to both the thousands of protecting groups covered by the '537 Patent's "azido group," and any embodiment where the phosphate bonding process connecting one nucleotide to the OH molecule of the next nucleotide at the 3′ position could be blocked via a protecting group that was not at the 3′ location, but across at its 2′ location. For both, QIAGEN presented unrebutted expert evidence explaining the amount of embodiments encompassed by the claims, the illogic of the embodiments to a skilled artisan, the complete lack of guidance or working examples in the '537 Patent, and the extensive experimentation therefore required to synthesize and test for nucleotides encompassed by the claims. But the lower court did not even cite

the correct legal standard, much less analyze the foregoing in light of it. Rather, the court adopted Illumina's position that QIAGEN's argument failed because QIAGEN did not have its expert conduct complex experiments or otherwise show data that these embodiments in fact would not work during the expedited and limited discovery period of a preliminary injunction proceeding. Appx15. This result turns the parties' respective burdens on their heads.

Third, the lower court committed both legal and factual error in concluding that QIAGEN had not raised a substantial question as to the obviousness of the '537 Patent's claims over prior art *Ju* or *Tsien* in combination with *Greene & Wuts*. The lower court held that if a skilled artisan knew of the azidomethyl protecting group used to block a 3′OH as disclosed in *Greene & Wuts*, she would be taught away from using it because she would conclude it could not be cleaved with high efficiency, as required by the SBS process described in *Ju* and *Tsien*. But this missed QIAGEN's argument and unrebutted expert testimony that (1) the claims of this patent do not require high cleaving efficiency (as this Court has ruled in a previous case involving this patent), (2) a person of skill would be motivated to use the labeling methods of *Ju* and *Tsien* with the *Greene & Wuts* azidomethyl protecting group for non-SBS embodiments that do not require high-cleaving efficiency, and (3) a skilled artisan would have used routine optimization methods to easily achieve high efficiency cleavage if motivated to use the

protecting group for SBS.

Fourth, the lower court committed clear error when it held that Illumina had met its burden to show a substantial threat of irreparable harm. Illumina's motion premised irreparable harm on the threat of lost sales and lower prices, but neither had happened 10 months after GeneReader's launch and, in any event, irreparable harm cannot be premised on quantifiable lost sales or insubstantial in-roads into a patentee's market share. The lower court concluded the market for selling "Next Generation Sequencing" ("NGS") sequencers to clinical labs was a new one, but the evidence was that the market was mature, and includes a number of competitors better situated to cause "harm" to Illumina than QIAGEN. The lower court found QIAGEN's pricing model to be "disruptive" to Illumina without any explanation of what that meant. While Illumina had a sales executive declare QIAGEN's pricing model may make Illumina change its own, the lower court ignored Illumina's admissions that (1) after 10 months it had not changed its pricing, nor had decided to do so in the future, (2) QIAGEN's product was the same price or more expensive than those of Illumina and other competitors, and (3) QIAGEN's "disruptive" pricing model was used by others in the market, including Illumina. Finally, the lower court did not address that after GeneReader being on the market for 10 months, Illumina continued to dominate over [redacted] of the market, while QIAGEN had less than [redacted]

In sum, the lower court abused its discretion when it granted a preliminary injunction based on errors of law and a fundamental misreading of the evidence concerning irreparable harm. In light of those errors, the court's ruling should be reversed, the preliminary injunction should be vacated, and the case should be remanded for a trial on the merits.

STATEMENT OF THE ISSUE

Did the lower court abuse its discretion when it granted Illumina's Motion for Preliminary Injunction and enjoined QIAGEN from selling its GeneReader Next Generation Sequencing DNA sequencer instrument in the U.S. where QIAGEN's challenges to the validity of the '537 Patent were correct as a matter of law and supported by unrebutted expert testimony, and where Illumina presented no evidence of irreparable harm?

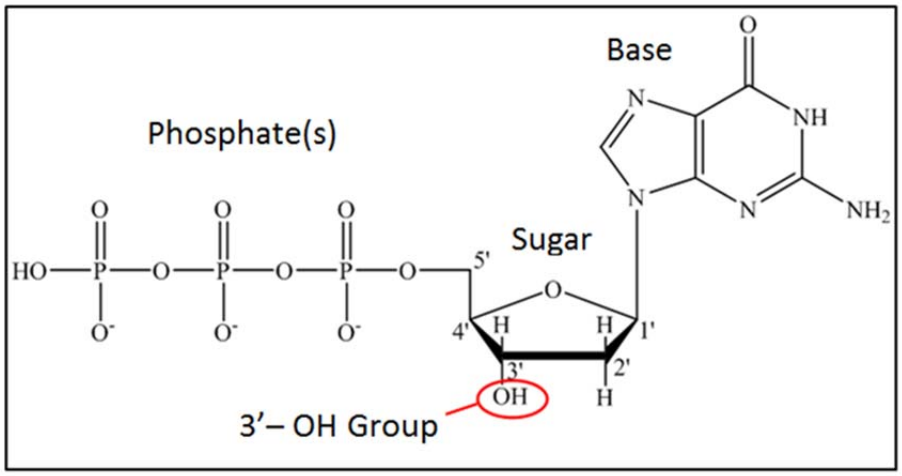
SUGGESTED ANSWER: YES.

STATEMENT OF THE CASE

I. "NEXT GENERATION SEQUENCING" OF DNA

A. DNA and Its Nucleotide Components

DNA contains the genetic information for all living organisms. DNA is made of "nucleotides" that are linked together. Each nucleotide is made up of three chemical groups (or "moieties"): a base (either adenine ("A"), thymine ("T"), guanine ("G"), or cytosine ("C")), a sugar (a ring of five carbon atoms), and one or more phosphate groups, Appx1080 at ¶¶ 28, 30, as shown here:

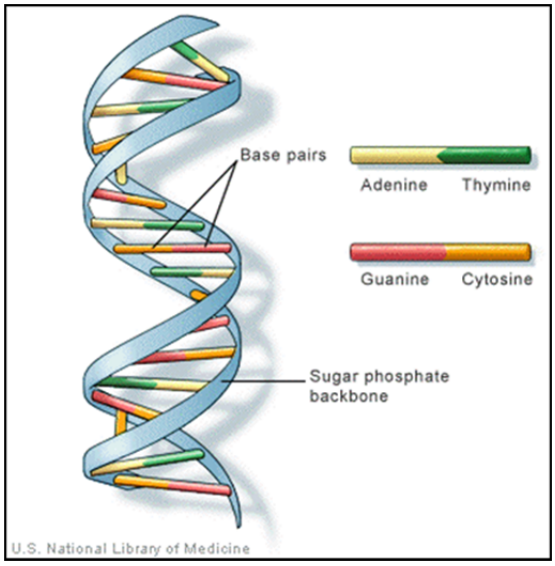


As stated, the sugar of a nucleotide contains five carbon atoms (shown above). By convention, the carbons are numbered one prime ("1′"), through five prime ("5′"). In DNA, the sugar is a deoxyribose with a hydroxyl group ("OH") attached at the 3′ position only, which is referred to as the "3′-OH group" (circled in red in the figure). Appx1080 at ¶ 31. The phosphate groups of nucleotides are attached to the 5′-carbon atom of the sugar. There can be nucleotides that have one ("mono-"), two ("di-"), or three ("tri-") phosphates. The nucleotides of a DNA chain are held together and elongated by adding, or "incorporating," one nucleotide to another via a bond that links the phosphate group at the 5′ location of one nucleotide to the 3′-OH group of the neighboring nucleotide. Appx1080-1081 at ¶ 32.

In addition to nucleo**tides**, there are similarly structured molecules called nucleo**sides**. Where nucleotides contain a base, a sugar, and phosphate groups, nucleosides contain only a base and a sugar, but no phosphate groups.

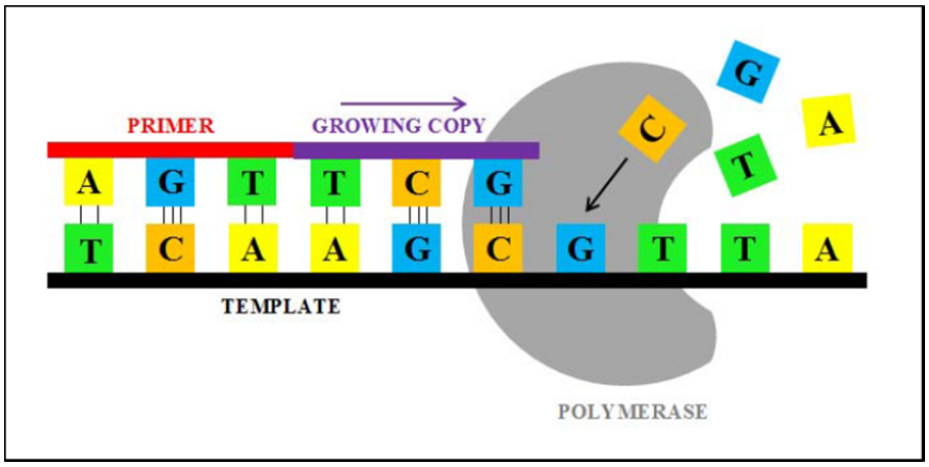
Because nucleosides do not contain phosphate groups, they cannot be incorporated into a growing DNA strand by a polymerase enzyme. Appx1083 at ¶ 36.

In nature, two chains of nucleotides form a double helix structure. The two chains are connected to each other by hydrogen bonds between complementary base pairs: A always with T, and G always with C. These pairs of nucleotides form the "rungs" in the DNA double helix "ladder," with the sugar-phosphate bonds forming the "rails" of the ladder, or the "backbone" of the double helix. Appx1081 at ¶ 33. A diagram of a DNA double helix is shown below:

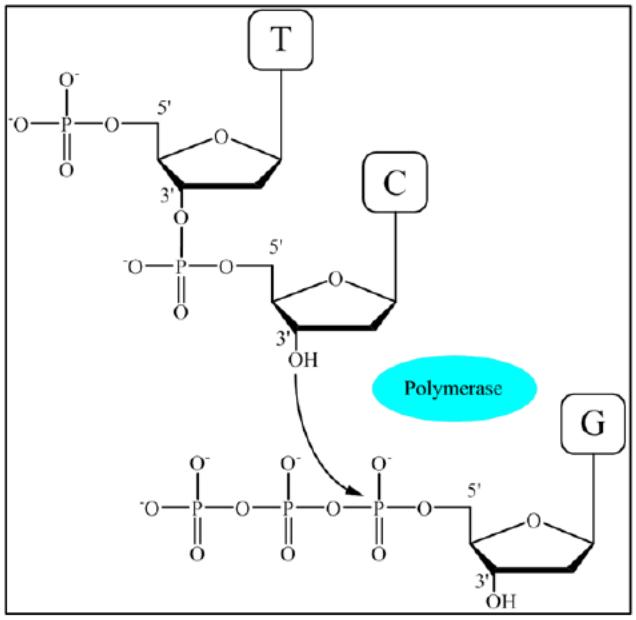


To replicate DNA, the two strands are unwound, the complementary bases separate, and then each of the two chains of nucleotides serves as a template to create the new strands. A short chain of nucleotides, called a primer, matches—and therefore binds to—corresponding nucleotides at the beginning of the DNA strand to be copied. An enzyme called a polymerase extends the primer along the

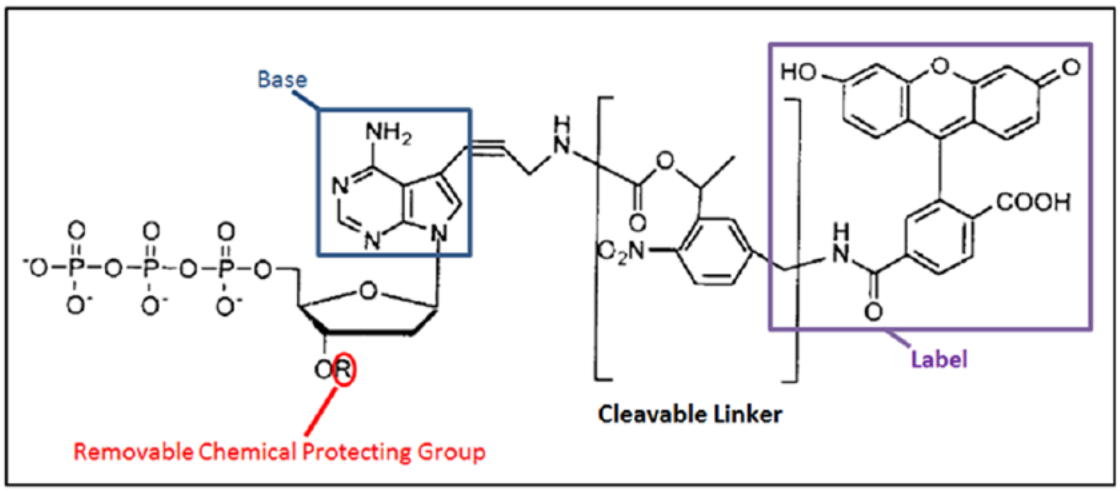
original template strand by adding nucleotides with bases that are complementary to those of the template in accordance with the base pair rules: A-T and G-C. Appx1081 at ¶ 34. For example, if the next nucleotide in the template to be copied has a "G" base, the polymerase enzyme would incorporate into the growing chain a nucleotide with a complementary "C" base:



In the illustration above, each square is a nucleotide. When the polymerase incorporates the next nucleotide on the growing strand, it does so by facilitating the formation of a bond between the phosphate group at the 5′ position of a free nucleotide and the 3′-OH group of the last nucleotide in the growing chain. For this reaction to take place, the last nucleotide in the chain ***must*** have a 3′-OH group available for bonding to the phosphate group at the 5 ′ position of the incoming nucleotide, as shown in the following diagram:



Sometimes, a person of skill in the art wants to stop this natural linkage reaction from taking place. Appx1084, at ¶ 39. To do this, artisans have known for a long time before the '537 patent that they can modify a nucleotide so that it has a removable "protecting group" that replaces the hydrogen atom in the 3′-OH group position of the sugar (the "R" group circled in red below):



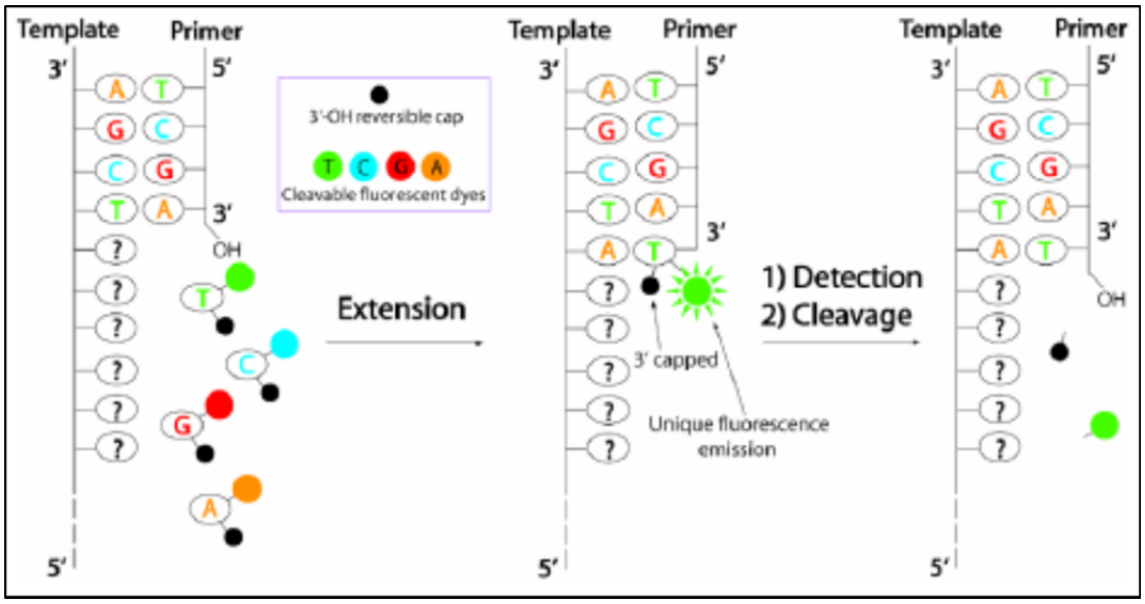
Appx1134 at Fig. 7 (annotations added). This protecting group "protects" or blocks the 3′-oxygen from forming a bond with the phosphate group of the next incoming nucleotide. Appx1084 at ¶ 39. It was known before the '537 Patent that such protecting groups should be small, to allow for the 3′OH protected nucleotide itself to be incorporated into the nucleic acid strand. Appx1090-1091 at ¶¶ 60-63; Appx1158 at 10:4-7. Once the incoming nucleotide with the protecting group is incorporated into the growing strand, the protecting group prevents the polymerase enzyme from incorporating additional nucleotides. If desired, skilled artisans knew how to remove this protecting group to allow for the incorporation of additional nucleotides. Appx1085-1086, ¶¶ 40-42. Persons of skill used these techniques before the priority date of the '537 Patent, often consulting the protecting group treatise, *Greene & Wuts*. Appx1086 at ¶¶ 43-44.

B. Modified Nucleotides, Methods of Labeling, and "Sequencing-by-Synthesis"

Before the disclosures made in the '537 Patent, the use and synthesis of "labeled" nucleotides, where a detectable label (such as a fluorescent label that would emit detectable light) would be attached to the nucleotide's base, was well known. Appx32, at 1:62-65; Appx1083, Appx1094-1095, at ¶¶ 37, 73 (citing Appx2329-2363). Skilled artisans also knew before the '537 Patent's priority date how to create and have incorporated into a growing nucleic acid chain a nucleotide with both a 3′-OH protecting group and a label. Moreover, the skilled artisan

understood that these nucleotides could be used both for methods that did not involve DNA sequencing (such as the synthesis and use of genetic "probes" or single strands of nucleotides), and for methods used to determine the sequences of the bases in DNA, known as "sequencing by synthesis," or "SBS". Appx1083-1086, Appx1090-1091, Appx1093-1096, at ¶¶ 37-41, 57-63, 70-75.

Ju and *Tsien* are examples of prior art that discuss methods of labeling a nucleotide with a removable 3′-protecting group in such a manner that it could be incorporated into a growing nucleic acid chain. *See* Appx1123-1235. *Ju* and *Tsien* both use this structure and method for the ultimate purpose of sequencing DNA by SBS, a sequencing method shown schematically in the figure below:



Ju and *Tsien* used their labeling methods for SBS, a person of skill interested in using labeled and protected nucleotides for non-SBS methods would know of SBS literature like Ju and Tsien, and would look to them for SBS and

non-SBS uses because he or she knew that SBS was a good place to look for guidance on how to incorporate labeled and 3′ protected nucleotides. Appx1093-1095, at ¶¶ 70-74.

Ju and *Tsien* taught to immobilize a DNA template on a solid surface, Appx1155 at 4:23-28; Appx1157 at 8:18-23; Appx1217 at 11:34-36, 32:9-33:19, 34:7-34, and then attach a primer to the DNA template to provide a starting point for growing the DNA strand. Appx1155 at 4:29-30, 8:24-25; Appx1217 at 11:34-36, 32:9-33:19, 34:7-34. The person of skill then would know to add a polymerase and one or more labeled, 3′-O-protected nucleotides. Appx1155 at 4:31-43; Appx1157 at 8:26-39; Appx1217 at 12:22-27. The polymerase would incorporate ***only one nucleotide*** because the 3′-OH protecting group prevents the addition of another nucleotide (*i.e.*, the protecting group terminates the polymerase reaction—the "Extension" step shown above). Appx1155 at 4:33-34; Appx1157 at 8:29-30; Appx1187. After incorporation of the labeled nucleotide, *Ju* and *Tsien* describe detecting the label—the "Detection" step shown above. Appx1155 at 4:46-49; Appx1157 at 8:42-45; Appx1217 at 13:1-13; Appx1206 at 31:1-35.

Ju and *Tsien* further explain how the resulting labeled nucleic acid molecule can be used in a SBS method. *Ju* and *Tsien* describe having a different label for each of the four possible nucleotides, to determine which base was incorporated. Appx1155 at 4:46-49; Appx1557 at 8:42-45; Appx1217 at 13:1-13;

Appx1206 at 31:1-35. After detection of the base, both *Ju* and *Tsien* teach removing the 3′-OH protecting group and the cleavable linker—the "Cleavage" step in the figure above, Appx1155 at 4:55-60; Appx1557 at 8:51-56; Appx1160 at 14:12-23; Appx1217 at 13:14-22; Appx1202 at 27:33-29:2, and then repeating the steps to sequence additional nucleotides. Appx1155 at 4:62-64; Appx1557 at 8:59-61; Appx1217 at 13:22-35.

Ju and *Tsien* both provide guidance as to what a person of skill should look for in a protecting group. Ju notes that the protecting group should be small (preferably a small ether), so that it would not interfere with the nucleotide's incorporation into the nucleic acid strand. Appx1158 at 10:4-7; Appx1157-1158 at 8:9-12, 10:1-7; Appx1195-1196. This guidance is relevant for both SBS and non-SBS labeling methods. Appx1093-1100, at ¶¶ 70-87. *Ju* and *Tsien* also gave guidance as to what would be needed in a protecting group used only for SBS—that the protecting group be removable with high efficiency (*i.e.*, that it is removed from all nucleotides), since SBS required accurately detecting which of four labels was incorporated, and then repeating the steps again. Appx1158 at 10:1-7; Appx1195-1196.

Thus, a skilled artisan using the methods of *Ju* and *Tsien* to synthesize DNA or a detectable probe would want a protecting group to be a small ether. However, while a person of skill knew how to optimize cleaving conditions to

increase efficiency (especially here, where much higher concentrations of cleaving reagent can be used because in Ju's and Tsien's methods, the DNA is attached to a solid surface rather than in solution), he or she would not need the protecting group to be cleaved with high efficiency when used for non-SBS methods such as those described in the claims of the '537 Patent. Appx1093-1095, Appx1098 at ¶¶ 71-74, 81.

C. "Azido" Protecting Groups for Creating Labeled Nucleic Acids

Before the '537 Patent, one of ordinary skill would have been aware that protecting groups that include an "azido" molecule (commonly shown as "N_3") were being used to protect OH groups. For example, the '537 Patent itself directs the public to the foremost protecting group treatise in the field of organic chemistry synthesis, *Greene & Wuts*, stating that "[s]uitable protecting groups will be apparent to the skilled person, and can be formed from any suitable protecting group disclosed in Green[e] and Wuts." Appx35 at 7:65-67. *Greene & Wuts* states that an "azidomethyl" group—a type of azido group with the chemical formula CH_2N_3—could be used to protect an OH group of a phenol molecule. Appx1506.

Azido groups also were used to block the 3′-OH group of a nucleotide before the '537 Patent. For example, *Tsien* describes the use of an azido group as part of a blocking modification to a nucleotide:

> . . . 3′-blocked dNTPs are used that can be incorporated in a template-dependent fashion and easily deblocked to yield a viable 3′-OH

> terminus. The most common 3′-hydroxyl blocking groups are esters and ethers. Other blocking modifications to the 3′-OH position of dNTPs include the introduction of groups such as -F, -NH_2, -OCH_3, -***N_3 [i.e., an azido group]***, -$OPO_3^{=}$, -$NHCOCH_3$, 2-nitrobenzene carbonate, 2,4-dinitrobenzene sulfenyl and tetrahydrofuranyl ether.

Appx1196 at ll. 9-17 (emphasis added).

II. THE '537 PATENT DISCLOSES USING AN AZIDO GROUP AS A BLOCKING GROUP FOR PURPOSES OF CREATING A LABELED NUCLEIC ACID

At issue in this appeal are claims 1-6, and 8 of the '537 Patent, titled "Labelled Nucleotides," which, as the title suggests, discloses methods that relate to labeled nucleotides. Appx32 at 1:14. In particular, claims 1-6, and 8 of the '537 Patent recite a method of labeling a nucleic acid molecule using labeled nucleotides or nucleosides. Appx41 at 19:49-20:18. As described in the '537 Patent, the claimed method has various uses in molecular biology. For example, the '537 Patent teaches that labeled nucleotides and nucleosides may be useful in techniques such as "polynucleotide synthesis, nucleic acid amplification, nucleic acid hybridization assays, single nucleotide polymorphism studies, and other techniques using enzymes such as polymerases, reverse transcriptases, terminal transferases, or other DNA modifying enzymes." Appx32-33, Appx36 at 2:3-11, 4:37-48, 10:33-41. In addition, like the '537 Patent's predecessors, *Ju* and *Tsien*, the labeling method of the '537 Patent can be used for SBS. Appx32-33, Appx35 at 1:47-67, 2:50-3:3, 3:60-62, 8:48-49.

Claim 1 (the only independent claim at issue in this case) is directed to a method of labeling a nucleic acid molecule by incorporating into the nucleic acid chain a nucleotide or nucleoside molecule:

> 1. A method of labeling a nucleic acid molecule, the method comprising incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule, wherein the nucleotide or nucleoside molecule has a base that is linked to a detectable label via a cleavable linker and the nucleotide or nucleoside molecule has a ribose or deoxyribose sugar moiety [a "moiety" is a sub-part within the overall molecule], wherein the ribose or deoxyribose sugar moiety comprises a protecting group attached via the 2′ or 3′ oxygen atom, and said protecting group can be modified or removed to expose a 3′ OH group and the protecting group comprises an azido group.

Appx41 at 19:49-59. Thus, claim 1 requires the incorporation of a nucleotide or nucleoside having (1) "a base that is linked to a detectable label via a cleavable linker," (2) "a ribose or deoxyribose sugar moiety" comprising "a protecting group attached via the 2′ or 3′ oxygen atom," that "can be modified or removed to expose a 3′-OH group," and (3) "the protecting group comprises an azido group." Appx41.

Claims 2-6, and 8 of the '537 Patent further limit the method of claim 1 in various ways. Claim 4 further limits the "nucleotide" option of claim 1, "wherein the nucleotide [embodiment in claim 1] is a deoxyribonucleotide

triphosphate," a type of DNA nucleotide that has three phosphate groups. Claim 6 limits the protecting group to an azidomethyl (CH_2N_3) group.[2]

III. THE NGS MARKET AND QIAGEN'S GENEREADER SEQUENCING INSTRUMENT

The Next Generation Sequencing market was approximately [redacted] in 2014, and Illumina owned [redacted] of that market. *See* Appx1626. Illumina estimated that its next-closest competitor—Thermo Fisher Scientific Inc.—had [redacted] of the NGS instrument market in 2014. Appx1626; Appx1117 at ¶ 6. In early 2015, Illumina projected the NGS market would grow from [redacted] in 2014, to approximately [redacted] in 2017, of which Illumina expected to capture almost 85%. Appx1626-1627. The U.S. represents approximately 40-50% of the worldwide NGS market. *See* Appx1117 at ¶ 5.

Illumina currently sells a number of "benchtop" sequencing instruments, and has offered benchtop products for several years, including its "MiSeq" sequencer. *See* Appx1055-1057 at ¶¶ 14-17. Illumina admits that clinical laboratories performing diagnostic testing have been a "key target" of Illumina's sales efforts for its benchtop sequencers for several years. Appx1057 at ¶ 18. For example, in 2013, Illumina's "MiSeqDX" became the first NGS system

[2] The limitations added in claims 2, 3, 5, and 8 are not relevant to this case.

to receive FDA approval, an approval designed to appeal to clinical laboratory customers. Appx1057 at ¶ 17; *see also* Appx1580-1581.

Interest in or use of NGS by clinical laboratories is nothing new—over the last several years, the clinical laboratory market made up a significant and growing percentage of Illumina's NGS business. *See* Appx1060 at ¶ 24.; *see also* Appx1632. Nor has this sector been shown to be "up for grabs"—Illumina dominates this sub-market, owning at least [redacted] of the market in 2014, with Thermo Fisher the second largest competitor. Appx1627; *see also* Appx1577-1578. Illumina has identified multiple other substantial competitors in the clinical laboratory market over the last two years. Appx1117 at ¶ 6; *see also* Appx1642; Appx1651. More recently, Illumina identified a number of start-up companies that it believes are likely to become competitors in the NGS market going forward. *See* Appx1682.

Illumina has remained confident in the stability of its dominant market position, even in the face of this increasing number of competitors in the clinical laboratory sub-market. In January 2015, Illumina stated that it expects its revenue share in NGS instruments and consumables to remain above [redacted] Appx1627. And in November 2015, immediately after the launch of GeneReader, Illumina told analysts that it did not expect GeneReader to make a "[redacted] on future benchtop instrument placements." *See* Appx1744.

QIAGEN launched the GeneReader on November 4, 2015. GeneReader is based on technology of IBS, which QIAGEN acquired in 2012. IBS has the exclusive license for Professor Ju's (of Columbia University) SBS patents discussed in this memorandum. Appx1117. Since 2012 GeneReader was further developed by IBS and other QIAGEN entities. *See* Appx1117 at ¶ 3. Before launching the product in November 2015, QIAGEN invested almost [redacted] in operational costs, capital expenditures, and employee time in developing GeneReader. Appx1121 at ¶ 29. QIAGEN invested an additional approximately [redacted] (half in the United States) to market GeneReader. Appx1121 at ¶ 30.

GeneReader employs SBS to sequence DNA samples, but does not have the raw sequencing power of Illumina's products. Appx1119 at ¶¶ 15-16. What sets GeneReader apart is that it is one of the first NGS products to offer a single platform for customers to engage in the entire NGS workflow, from sample preparation to data analysis, *see* Appx1119 at ¶ 18, along with the flexibility to load multiple sequencing runs at varying times, Appx2728. At the time of the Preliminary Injunction Order, GeneReader only could sequence tumors for one of twelve genes on a particular "cancer panel" QIAGEN offers—the "Actionable Insights Tumor Panel." Appx1119 at ¶ 15. In contrast, customers can use the Illumina (and Thermo Fisher) NGS instruments to sequence anything they want.

GeneReader is similarly priced to Illumina's benchtop sequencer, and more expensive than Illumina's newest instrument. In addition to selling the GeneReader outright, and thereafter selling customers nucleotides and other "consumables" needed to run the sequencing tests on an as needed basis, QIAGEN also offers a program where the customer does not buy the GeneReader upfront, but "rents" it—the customer commits to buying a set quantity of consumables (or to doing a set number of tests using such consumables) per year, for a multi-year period, with the price of the GeneReader amortized into the price of the consumables. Appx1120-1121 at ¶¶ 22-23. This pricing model is not new or "disruptive"—both Illumina and Thermo Fisher have used it in the NGS market over the years. Appx1588-1591.

As of the date of the Preliminary Injunction Order (September 9, 2016), QIAGEN had sold [redacted] GeneReader instruments in the U.S. from when the product was launched in November 2015, Appx1118 at ¶ 7; all but one of the U.S. sales was a conditional sale that gives the customers a period of time during which they can test and validate GeneReader before they are committed to pay for the product. Appx1121 at ¶ 24. Before entry of the Preliminary Injunction Order, QIAGEN projected to sell another [redacted] GeneReaders worldwide in the remainder of 2016 and in 2017, about 40-50% of which would be in the U.S. Appx1117-1118 at ¶¶ 5, 11.

IV. ILLUMINA'S HISTORY OF LITIGATING THE '537 PATENT AND ITS REQUEST FOR PRELIMINARY INJUNCTION

A. The Delaware Litigation and *Inter Partes* Review of the '537 Patent and Related Patents

Illumina first asserted the '537 Patent against QIAGEN in 2012 in the United States District Court for the District of Delaware, against IBS (a QIAGEN subsidiary). IBS (and Columbia University) filed suit against Illumina, alleging infringement of several patents relating to SBS DNA sequencing technology, in a case captioned *Trustees of Columbia University in the City of New York v. Illumina, Inc.*, No. 12-cv-0376 (U.S.D.C. D. Del.) (Sleet, J.) (the "Delaware Litigation"). In response to that complaint, Illumina alleged patent infringement counterclaims of its own against IBS and QIAGEN, N.V. One of the patents that Illumina asserted against IBS in the Delaware Litigation was the '537 Patent. *See* Appx1000, Appx1021-1022.

The parties engaged in discovery in the Delaware Litigation, but then stipulated to a stay while they pursued *inter partes* review petitions of each other's patents before the Patent Trial and Appeals Board (the "PTAB," or the "Board"), and any subsequent appeals to this Court.[3]

[3] Two of the *inter partes* review proceedings (IPR2013-00128 and IPR2013-00266) involved Illumina patents in the same family as the '537 Patent, both of which included claims reciting modified nucleotides with a 3′-OH protecting group. Both proceedings resulted in the Board finding the relevant claims invalid

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On August 19, 2013, IBS filed a petition with the PTAB challenging the patentability of claims 1-6, and 8 of the '537 Patent. Among other grounds, IBS asserted that claims 1-6, and 8 were obvious (1) in view of *Ju* or *Tsien* in combination with *Zavgorodny*, and (2) in view of *Ju* or *Tsien* in combination with *Greene & Wuts*. *See Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, IPR2013-00517, 2014 WL 1253074, at *3 (P.T.A.B. Feb. 13, 2014) (Institution Decision). The Board instituted the IPR on the combination of *Ju* or *Tsien* with *Zavgorodny*. *Id.* at *9. The Board declined to institute an IPR on the combination of *Ju* or *Tsien* with *Greene & Wuts*, finding the combinations "redundant" with the combinations of art the Board already agreed to consider in the IPR. *Id.*

During the IPR, Illumina argued that *Zavgorodny*'s azidomethyl group would not have been suitable for use in SBS because the specific conditions disclosed by *Zavgorodny* for cleaving the azidomethyl protecting group would have been too inefficient for use in a sequencing reaction. *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1364-65 (Fed. Cir. 2016). The Board concluded as the sole reason for not invalidating the challenged claims that an ordinary artisan would not have considered it obvious to use *Zavgorodny*'s

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as obvious over *Ju* and *Tsien*, and this Court affirmed those judgments on appeal. *See Illumina Cambridge Ltd. v. Intelligent Bio-Systems, Inc.*, 638 Fed. Appx. 999 (Fed. Cir. 2016).

azidomethyl group as the 3′-OH protecting group in *Tsien*'s or *Ju*'s processes, because a skilled artisan would not expect that it could be removed quantitatively. *Id.*

Based on the PTAB's procedural rules, the Board did not consider arguments in IBS's reply memorandum in the IPR that a skilled artisan would have combined *Ju* or *Tsien* with *Zavgorodny* for methods within the scope of the '537 Patent's claims for ***purposes other than SBS*** that do ***not*** require high cleaving efficiency, or that a skilled artisan could have modified *Zavgorodny's* conditions to achieve quantitative cleavage. *Id.* at 1366. And, in fact, the Board excluded IBS's evidence submitted with its reply supporting the argument that a skilled artisan would have understood *Zavgorodny*'s cleaving conditions indeed to be "quantitative." *Id.*

On appeal of the PTAB's decision regarding the '537 Patent, this Court agreed that the Board erred in finding that the '537 Patent was valid because azidomethyl might not be cleaved quantitatively: "[I]t is of no moment that *Zavgorodny*'s protecting group would not be removed quantitatively in *Tsien* or *Ju*'s sequencing methods—***removal is simply not required by the claim of the '537 Patent.***" *Id.* at 1367 (emphasis added). The Court nonetheless affirmed the outcome of the IPR because it concluded that, while the '537 Patent's claims are broader than SBS and do not require high cleaving efficiency, IBS first relied on

SBS as the basis for a motivation to combine *Zavgorodny* with *Tsien* or *Ju*. *See id.* at 1368. In affirming the Board's procedural ruling to exclude the evidence and arguments in IBS's reply memorandum to the PTAB, the Court also declined to consider IBS's evidence that a skilled artisan would be motivated by non-SBS uses or that she would not have been deterred from using azidomethyl as a blocking group for SBS. *Id*. at 1370.

* * *

Although the parties agreed to a stay of proceedings in the Delaware Litigation while the IPR occurred, they further agreed to allow certain fact discovery during the stay. *See* Appx2745-2749. Among the discovery Illumina obtained in the Delaware Litigation was the structure of the modified nucleotides used in QIAGEN's GeneReader. On November 10, 2015, less than a week after QIAGEN launched GeneReader, Illumina demanded updated information regarding GeneReader's nucleotides. *See* Appx2738-2743. IBS produced the requested information on December 29, 2016. Appx2739. Once it sought and obtained the requested information about GeneReader in the Delaware Litigation, Illumina did nothing more until filing its new lawsuit in the Northern District of California.

B. The New '537 Patent Preliminary Injunction Action in the Northern District of California

Over six months after QIAGEN launched GeneReader, on May 24, 2016, Illumina filed a complaint and motion for preliminary injunction in the Northern District of California, for the second time alleging infringement of the '537 Patent by IBS, and by other QIAGEN defendants. *See* Appx57. After expedited discovery and QIAGEN's opposition, supported by declarations from Dr. Michael Metzker and Mr. Jonathan Arnold, Appx1071-1122, the lower court held a short oral argument on August 25, 2016, Appx2094-2130, and, on September 9, 2016, it granted Illumina's motion for a preliminary injunction. Appx1-19.

First, the lower court concluded that QIAGEN did not raise a substantial question as to whether the '537 Patent was enabled. With regard to lack of enablement based on Illumina's claims improperly including a "nucleoside" embodiment, as well as a "nucleotide" embodiment, the court did not take issue with QIAGEN's expert's unrebutted opinion that a nucleic acid molecule without phosphate groups ***cannot*** be incorporated by a polymerase enzyme (as required by the claims). Nevertheless, it construed "nucleoside" as, in effect, the same as a nucleotide, because it is a molecule that ***could be*** "combined with phosphate groups." Appx13-14. In other words, the lower court decided, without any supporting evidence, that the claim term "nucleoside" (which is not operable and is

not enabled) could be transformed into a "nucleotide" (which is operable and should be deemed enabled), even though the '537 Patent's specification and claims uniformly refer to those molecules as different.

The lower court also decided there was no substantial question as to the enablement of Illumina's "azido group" and "2′" limitations, despite the absence of any meaningful guidance in the patent. The lower court conceded the "azido group" limitation "encompasses a broad class of more than one thousand chemical structures" that are not described in the '537 Patent. Appx4. As to enablement of a claim requiring a protecting group at the 2′ location to protect a process occurring across the sugar at the 3′ location, the lower court did not address QIAGEN's arguments that simply saying the protecting group would have to be big or of a certain characteristic to work from the 2′ location is not sufficient guidance, especially where *Ju* and *Tsien* instructed to keep protecting groups ***small*** so as to allow for incorporation. Rather, for both limitations, the lower court ruled against QIAGEN because it did not have, or develop with its expert, affirmative evidence that the embodiments "would not work," and for providing only "conclusory" statements from the expert that undue experimentation would be required. Appx15-16.

The lower also found no substantial question as to the obviousness of the claims. QIAGEN argued that the claims of the '537 Patent are obvious because

a skilled artisan would have been motivated to combine the azidomethyl protecting group found in *Greene & Wuts* with the modified nucleotides disclosed by *Ju* or *Tsien*. The lower court believed that, because *Ju* and *Tsien* concerned "aliphatic alcohols" (such as DNA), while *Greene & Wuts* disclosed azidomethyl in the context of phenols, the skilled artisan would not have thought to consider azidomethyl as a protecting group. Appx10-11. The court also ignored QIAGEN's non-SBS motivation argument, finding that, even if a person of skill did know of *Greene & Wuts*' disclosure of azidomethyl, he or she would not believe from the treatise that azidomethyl could be cleaved with high efficiency, as demanded by *Ju* and *Tsien* for SBS methods. Finally, even though it found that a skilled artisan would not look to *Greene & Wuts* to use an azidomethyl protecting group, the lower court found the azidomethyl claim (claim 6) did not suffer from lack of written description. It so found despite ***the only guidance*** in the '537 Patent to choose azidomethyl from over 1,000 azido groups is that "suitable protecting groups will be apparent to the skilled person, and can be formed from any suitable protecting group disclosed in *Green[e] and Wuts*," with "examples of ***such*** protecting groups [in *Greene & Wuts*] shown in Figure 3." Appx35 at 7:65-67, 8:1 (emphasis added).

The lower court also concluded that Illumina would suffer irreparable harm if it did not issue the preliminary injunction banning QIAGEN from further

promoting or selling GeneReader in the U.S. The court did not address the evidence concerning the parties' respective sales projections and market shares in the coming years, but instead adopted Illumina's assertions in its motion papers that Illumina "views Qiagen's products as a threat and expects Qiagen to become a more significant threat over the next two years absent an injunction." Appx17. The court found that Illumina has demonstrated a "real risk that Qiagen could capture and redefine the market," Appx18, despite QIAGEN's sales of only eight instruments in the ten months before the Preliminary Injunction Order. Again, there was no expert testimony, no market survey data, and no other evidence that supported the existence of this "real risk."

On September 15, 2016, after requesting leave of the lower court, QIAGEN filed a motion to clarify and to stay the Preliminary Injunction Order pursuant to Federal Rule of Civil Procedure 62(c). Appx63. QIAGEN's motion requested: (a) a clarification of the Preliminary Injunction Order to ensure that QIAGEN could continue to manufacture the GeneReader in the U.S. for sale and use outside the U.S.; (b) a stay of the Preliminary Injunction Order as to existing U.S. GeneReader customers, to permit QIAGEN to continue to supply such users with the modified nucleotide consumables; and (c) a stay of the Preliminary Injunction Order in its entirety. Appx20-23.

On September 22, 2016, the lower court granted QIAGEN's motion in part. It clarified that the Preliminary Injunction Order permitted QIAGEN to continue to manufacture GeneReader in the U.S., stayed the Injunction Order as to existing U.S. GeneReader users, but denied QIAGEN's request to stay the Preliminary Injunction Order in its entirety. Appx20-23. In denying the motion to stay in its entirety, the court recapitulated its belief that "it remains unlikely that Qiagen will succeed in clearly and convincingly showing that the '537 Patent is obvious," and that there is a risk of irreparable harm to Illumina. Appx21. The lower court also further addressed QIAGEN's argument that a skilled artisan would have a non-SBS motivation to combine *Greene & Wuts* that would not require high cleaving efficiency, noting supposed deficiencies in QIAGEN's expert's declaration. *See* Appx21.

QIAGEN filed its Notice of Appeal the next day, on September 23, 2016. Appx93. The appeal was docketed on September 27, 2016. On September 30, 2016, QIAGEN filed a motion to expedite the briefing schedule in this appeal, as well as a motion to stay the Preliminary Injunction Order pending appeal. Dkt. Nos. 9-12. On October 3, 2016, the Court set a briefing schedule on those motions and directed that QIAGEN file this opening brief by October 14, 2016. Dkt. No. 13.

SUMMARY OF THE ARGUMENT

The lower court erred in granting Illumina's motion for a preliminary injunction in this matter. The court's conclusion that QIAGEN had failed to raise a substantial question as to enablement was based on a number of different legal errors, and its conclusions as to obviousness failed to address expert evidence QIAGEN submitted that Illumina did not rebut with its own expert. The lower court further erred in relying solely on a conclusory declaration from an Illumina sales executive in determining that Illumina faced at substantial threat of irreparable harm absent an injunction. Other than Illumina's self-serving declaration, no evidence supported the conclusion that the GeneReader was a threat to take substantial market share from Illumina during the likely pendency of this case. The only evidence of record was contrary to the lower court's conclusion that GeneReader might "perform poorly," and thereby harm the market for NGS sequencing. Having based its ruling on both legal and factual errors, the lower court should be reversed and the Preliminary Injunction Order should be vacated.

ARGUMENT

A preliminary injunction is a "drastic and extraordinary remedy that is not to be routinely granted." *Nat'l Steel Car, Ltd. v. Canadian Pac. Ry., Ltd.*, 357 F.3d 1319, 1324 (Fed. Cir. 2004) (internal quotation marks omitted) (quoting *Intel*

Corp. v. ULSI Sys. Tech., 995 F.2d 1566, 1568 (Fed. Cir. 1993)). As the party seeking a preliminary injunction, Illumina had the burden to show that: (1) it was likely to succeed on the merits; (2) it was likely to suffer irreparable harm if the injunction is not granted; (3) the balance of hardships weighed in its favor; and (4) an injunction was in the public interest. *Apple Inc. v. Samsung Elecs. Co.*, 695 F.3d 1370, 1373-74 (Fed. Cir. 2012).

Although the grant or denial of a preliminary injunction is reviewed for abuse of discretion, if the decision to grant the injunction flowed from legal error, reversal is required. *Jack Guttman, Inc. v. Kopykake Enters., Inc.*, 302 F. 3d 1352, 1356 (Fed. Cir. 2002). The lower couer's findings of fact in support of its preliminary injunction are reviewed for clear error. *Abbott Labs. v. Andrx Pharms., Inc.*, 452 F.3d 1331, 1335 (Fed. Cir. 2006).

I. THE LOWER COURT'S CONCLUSION THAT ILLUMINA IS LIKELY TO DEFEAT QIAGEN'S INVALIDITY ARGUMENTS IS LEGAL ERROR

A. The '537 Patent's Claims are Not Enabled

The enablement language of Section 112 requires that "the specification of a patent . . . teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation." *In re Wright*, 999 F.2d 1557, 1561 (Fed. Cir. 1993) (internal quotations omitted).

1. Claims 1-6, and 8 are Invalid as Not Enabled Because a Nucleoside is Not Operable in the Claimed Methods

The '537 Patent's claims are invalid because they recite an inoperative

embodiment. If a claim is shown to be inoperative, then it necessarily fails to meet the how-to-use aspect of the enablement requirement. *In re Swartz*, 232 F.3d 862, 863(2000).

a. As Defined in the '537 Patent, a Nucleoside Without Phosphate Groups is Inoperable

The '537 Patent's claims are broad and encompass the incorporation of two alternative molecules: a "nucleo**tide** or nucleo**side** molecule." It is well-established law that, when a claim contains such alternative language, the patentee must enable both alternatives. *Application of Fouche*, 439 F. 2d 1237, 1242 (C.C.P.A 1971) (affirming rejection under the "how-to-use" provision of § 112, "since if such compositions are in fact useless, appellant's specification cannot have taught how to use them."); *see also Sitrick v. Dreamworks, LLC*, 516 F. 3d 993, 1000 (Fed. Cir. 2008) (affirming invalidity for lack of enablement because the specification did not explain how one of two alternative embodiments encompassed by the claims would work).

Here, Illumina's nucleoside alternative fails the test of enablement. As recognized by the experts of both parties, the '537 Patent itself states that a "nucleoside" is different than a "nucleotide," in that a nucleoside does not have phosphate groups, while a nucleotide does. *See* Appx33, col. 4, ll. 59-60; Appx1088, at ¶ 49; Appx1035, at ¶ 46. Again, as experts from both parties agree, there is no way that a nucleoside can work for the methods claimed in the '537

Patent, because it has no phosphate groups. *See* Appx1102, at ¶ 95; Appx1748-1753, at Appx1753. Moreover, there is ***no*** guidance in the '537 Patent that otherwise explains how a ***nucleoside*** would work for the claimed methods.[4]

Despite this, the lower court found the patent likely enabled. The court construed the term "nucleoside" as being the same as a "nucleotide," because a person of skill supposedly would understand "nucleoside" to be a molecule that did not have phosphates, but that still could be combined with phosphate groups. Appx13-14.

By wrongly construing "nucleoside" to encompass phosphate groups, the lower court committed legal error. The lower court's construction of nucleoside is in direct conflict with the definition of "nucleoside" Illumina used in the '537 Patent. This justifies reversal of the preliminary injunction order. *Jack Guttman*, 302 F.3d at 1361-62 ("grounding a decision on a preliminary injunction on a claim construction at odds with an unambiguous definition in the intrinsic evidence constitutes an abuse of discretion"). The lower court's construction also

[4] Illumina's actions in one of the IPR proceedings between the parties, involving a patent from the same family as the '537 Patent, U.S. Patent No. 7,057,026, shows that Illumina itself understands nucleosides are not enabled for the methods disclosed in the '537 Patent. In the IPR, Illumina sought to amend its claims to, among other things, replace the phrase "a nucleotide or nucleoside molecule" with "a nucleotide triphosphate molecule." *See Illumina Cambridge, Ltd.*, 638 Fed. Appx. at 1002.

improperly strips the "nucleoside" limitation of any meaning at all. *See Texas Instruments Inc. v. U.S. Int'l Trade Comm'n*, 988 F.2d 1165, 1171 (Fed. Cir. 1993) (rejecting claim construction that would "read an express limitation out of the claims").

Moreover, the lower court erred by relying on extrinsic evidence that is contrary to the explicit definition of nucleoside in the '537 Patent. *Ruckus Wireless, Inc. v. Innovative Wireless Solutions, LLC*, 824 F.3d 999, 1003 (Fed. Cir. 2016) ("[l]egal error arises when a court relies on extrinsic evidence that contradicts the intrinsic record"); *Northern Telecom Ltd. v. Samsung Elecs. Co.*, 215 F.3d 1281, 1288 (Fed. Cir. 2000). The lower court found that "a skilled artisan might alternatively have referred to a nucleotide as a nucleoside moiety combined with a phosphate group." Appx13. Even if this generally may be true for a skilled artisan, this observation ***is not true*** of the '537 Patent, which refers to the molecules separately.[5] Thus, it was legal error for the lower court to rely on extrinsic evidence to change the definition of "nucleoside" in the '537 Patent.

[5] The lower court stated that if the '537 Patent's claims were limited to nucleotides, one could evade infringement by describing the patent's nucleotides as "nucleoside triphosphates." *See* Appx13-14. That is not the case. "Nucleoside triphosphates" and "nucleosides" are different terms meaning different things. A nucleoside triphosphate would meet the definition of a nucleotide in the '537 patent and thus would not evade infringement.

In any event, the extrinsic evidence relied on by the lower court—Dr. Metzker's patent application that uses the same "nucleotide or nucleoside" claim language—does not support the court's claim construction. Appx13-14. Under cross examination by Illumina's counsel regarding his 200-page patent application, Dr. Metzker testified that his claim may have the same problem as the '537 patent because it recites the incorporation of a "nucleotide or nucleoside." But, in fact and contrary to the lower court's conclusion and Illumina's cross examination questioning, Dr. Metzker's application ***does not have the same problem*** as the '537 Patent because the application specifically defines nucleosides as including "mono, di, and triphosphates." Appx1894-2093, at Appx1904. ***The '537 Patent contains no analogous language***.

The lower court broke with the explicit definition in the '537 Patent when it construed "nucleoside" as potentially including a nucleoside "combined with phosphate groups," Appx14, *i.e.*, a nucleotide. This construction was legal error. Because a nucleoside molecule is inoperable in the claimed methods, and because a patent cannot enable an inoperable embodiment, claims 1-6, and 8 of the '537 Patent are invalid as not enabled.

b. Claim 4 Incorporates the Option to Use a Nucleoside and Therefore is Not Enabled

The lower court held that, even if QIAGEN's nucleoside argument was right, it would not invalidate ***all*** claims of the '537 Patent. That was because

the court construed claim 4, which depends from claim 1, as eliminating the "nucleoside" option. Appx14. This was legal error.

Claim 4 reads as follows:

> The method of claim 1, wherein ***the nucleotide*** is a deoxyribonucleotide triphosphate.

Appx41 (emphasis added). The lower court held that this claim eliminated the alternative option to incorporate a "nucleoside" that is present in claim 1. However, this conclusion disregards the plain language of the claim, which says only that the "nucleotide" of claim 1 is now a deoxyribonucleotide triphosphate. For the lower court's view to be correct, claim 4 would have to be written "wherein ***the nucleotide or nucleoside*** is a deoxyribonucleotide triphosphate." But it wasn't. Because claim 4 was written as further defining only "nucleotide," it should not be construed as eliminating the nucleoside embodiment.[6]

Indeed, the lower court's construction is incorrect as a matter of law because it conflicts with 35 U.S.C. § 112(d). That section explicitly provides that a dependent claim must be construed ***to incorporate all the limitations*** of the independent claim to which it refers "and specify a ***further*** limitation to the subject matter claimed." *See* 35 U.S.C. § 112(d) (emphasis added). Claim 4 specifies a

[6] Illumina first made this argument as to the construction of claim 4 at oral argument on the injunction motion, Appx2116, and did so without evidentiary support. *Id*.

"further limitation" to the "nucleotide" portion of claim 1, but provides ***no*** indication that it limits the alternative in claim 1 to prevent use of a "nucleoside." The lower court concluding otherwise was an error of law.

2. The '537 Patent Does Not Enable the "2′" and "Azido Group" Limitations

The lower court abused its discretion in finding that QIAGEN's other enablement arguments are not likely to succeed. The '537 Patent claims are not enabled unless its specification "teach[es] those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation." *In re Wright*, 999 F.2d at 1561. Factors to determine whether a disclosure would require a skilled artisan to engage in undue experimentation include: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988).

a. The Lower Court Erred by Not Applying the *Wands* Factors

QIAGEN presented unrebutted expert testimony that the '537 Patent does not support the full scope of the claims. First, QIAGEN presented evidence that the claims' limitation for attachment of a protecting group "via the 2′ or 3′

oxygen atom" was not enabled as to the 2′ oxygen atom. Second, QIAGEN presented evidence that the limitation that the protecting group comprises an "azido group"—in each claim except claim 6—was not enabled as to its full scope. The lower court did not even attempt to apply the *Wands* factors to weigh the lack of guidance and working examples against the unpredictable nature of the claimed invention, the state of the prior art, and the breadth of the claims. Had it done so, it would have been clear that the '537 Patent does not provide adequate teachings to practice the full scope of the claims without undue experimentation.

2' Protecting Groups. The '537 Patent fails to enable this embodiment because the '537 Patent provides little to no guidance on how to use 2' protecting groups in the claimed methods of labeling. The only guidance for choosing a 2′ protecting group in the '537 Patent is that "the protecting [should be] of sufficient size or charge to block interaction at the 3′ position." Appx35 at 7:60-63. However, the '537 Patent does not provide even a single example of a protecting group that could be attached at the 2′ position and yet block the 3′ position of the molecule, where the phosphate group of the next nucleotide would bond, as required by the claimed methods. Appx1104 at ¶ 102. The '537 Patent also lacks examples of any RNA polymerases, or guidance for selecting one, which is the type of polymerase that would be required for nucleotides with 2′ protecting groups. Appx1104 at ¶¶ 102-03. Moreover, there was no guidance in the prior art

to cure these shortcomings. Unlike 3′ protecting groups, there is absolutely no evidence of record that 2′ protecting groups have ***ever*** been used to block interactions at the 3′ position, as the '537 Patent claims require.

This lack of guidance is particularly crippling to the enablement of these claims because, what little guidance there is regarding 2′ protecting groups, ***contradicts*** the '537 Patent's other teachings, and the prior art. Choosing a protecting group of sufficient size to block interactions at the 3′ position conflicts with the '537 Patent's (and the prior art's) teaching that nucleotides with large protecting groups are "difficult to incorporate onto an existing polynucleotide, due to an inability to fit into the polymerase enzyme active site." Appx32 at 1:55-61; *see also* Appx1103 at ¶ 100. Conversely with regard to claim 6, the '537 Patent does not teach a person of skill how azidomethyl (a small protecting group) would be of sufficient size to block interactions with the 3′-position. Appx1105 at ¶ 105). Other than saying during oral argument that QIAGEN did not show a 2′ protecting group would ***not*** work in the claimed method, Illumina offered no rebuttal to this argument—no expert testimony, no citation to relevant prior art.[7]

[7] Like the nucleoside issue, Illumina recognized this is a problem for the validity of its patents in the related IPR proceedings. *See Illumina Cambridge, Ltd.*, 638 Fed. Appx. at 1002 (showing Illumina's proposed amended claim eliminating "2′").

Azido Protecting Groups. The '537 Patent fails to enable the full scope of this limitation, which encompasses ***thousands*** of azido protecting groups, including ones much larger than the azidomethyl group taught in *Greene & Wuts*. Like with the 2' limitation, there is little guidance in the '537 Patent for using azido protecting groups. The '537 Patent does not provide a single example of a nucleotide that is incorporated with an azido protecting group. In fact, the '537 Patent specification does not even identify a single azido protecting group that would work in the claimed methods. Given the absence of any guidance in the '537 Patent, a skilled artisan would need to engage in undue experimentation to determine which of the azido molecules would work in the claimed method. Appx1102-1104 at ¶¶ 97-100.

QIAGEN introduced expert testimony that a skilled person would have had to synthesize and screen thousands of combinations of potential protecting groups in order to determine which protecting groups would work in the claimed methods. Appx1103-1104 at ¶¶ 99-100. As the prior art indicated, nucleotides with large protecting groups would not be incorporated by a polymerase into a nucleic acid molecule. Appx1103-1104 at ¶ 100 ("Welch explained that the 3'-*O*-blocked nucleotides with large protecting groups "tend to be too big to fit into the active site of DNA polymerase"). And there is no question that the azido group includes many relatively large molecules. *Id.* Dr. Metzker's

testimony, based on his knowledge of relevant prior art, was more than sufficient at the preliminary injunction stage to raise a "substantial question" regarding enablement and, accordingly, the validity of the '537 Patent. *Wyeth & Cordis Corp. v. Abbott Labs.*, 720 F.3d at 1385 (holding claims not enabled where skilled person would have to "synthesize and screen each of at least tens of thousands of candidate compounds"). Illumina did not submit any expert evidence to the contrary.

b. The Lower Court Erred by Requiring QIAGEN to Provide Experimental Evidence of Inoperative Embodiments

Despite the complete lack of any guidance in the '537 Patent, the lower court rejected QIAGEN's arguments. While acknowledging that "the number of combinations encompassed within the claims is significant," the court held that QIAGEN had not raised a substantial question at the preliminary injunction stage because it had "failed to identify *even one* inoperative combination." Appx15-16 (emphasis in original). This conclusion represents a fundamental misunderstanding of the law of enablement and flips the standard on its head.

Enablement is not about whether the claims encompass inoperative embodiments, but about whether the ***patent provides sufficient guidance*** to permit a person of skill in the art to make and use the claimed invention. *See Wyeth &*

Cordis Corp. v. Abbott Labs., 720 F.3d 1380, 1384 (Fed. Cir. 2013). While not irrelevant, the district court erred by ***requiring*** QIAGEN to provide experimental evidence of inoperative embodiements in opposing a preliminary injunction. As this Court's cases make clear, the fact that a claim encompasses a significant number of inoperative combinations is an indication that a skilled artisan would be required to engage in undue experimentation to practice the full scope of the claims. *See*, *e.g.*, *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1576 (Fed. Cir. 1984). But identifying particular inoperative embodiments ***is not a requirement***, particularly at the abbreviated preliminary injunction stage. *See Abbott Labs.*, 452 F.3d at 1335 (stating that a validity challenge at the preliminary injunction stage "may raise substantial questions of invalidity, on evidence that would not suffice to support a judgment of invalidity at trial"). Such evidence often comes from the ***patentee's experiments***, evidence QIAGEN can hardly be faulted for not presenting at the preliminary injunction stage. *See AK Steel Corp. v. Sollac*, 344 F.3d 1234, 1244-45 (Fed. Cir. 2003); *Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1372-73 (Fed. Cir. 1999). The lower court essentially created a rule that, to challenge a broad claim for enablement, the challenger must provide experimental evidence that the claim encompasses an inoperative embodiment. That conclusion has no support in the law and was legal error.

B. The '537 Patent is Invalid as Obvious Over *Ju* or *Tsien* in Combination with *Greene & Wuts*

As Illumina did not and could not contest that *Ju* or *Tsien* in combination with *Greene & Wuts* teaches all the limitations of claims 1-6, and 8 of the '537 Patent, the lower court focused on the motivation to combine the references. The lower court's conclusions were error for at least three reasons.

1. The Lower Court Erred in Concluding That a Person of Skill Would Not "Look To" the Azidomethyl Disclosure in *Greene & Wuts*

Central to the district court's conclusion that the '537 Patent is not obvious was its view that a skilled artisan would not "look to" the disclosure of azidomethyl in *Greene & Wuts* because that disclosure is in a chapter of the treatise covering phenols (chapter 3), rather than the chapter covering aliphatic alcohols like DNA (chapter 2). Appx11. This was wrong as an initial matter because a skilled artisan is deemed to know ***all*** the analogous art, and even Illumina never argued that references disclosing protecting groups were not analogous just because they pertained to phenolic hydroxyl groups. *E.g.*, *In re Carlson*, 983 F.2d 1032, 1038 (Fed. Cir. 1992). Nor could such an argument succeed, as the '537 Patent's guidance on selecting protecting groups simply directs the skilled person to "any suitable protecting group disclosed in Green[e] and Wuts," without specifying particular chapters. Appx35 at 7:65-67. Thus, the issue before the lower court was not whether a skilled artisan would have known of

the azidomethyl disclosure in *Greene & Wuts*, but whether he or she would have been motivated to use it in the SBS methods disclosed by *Ju* and *Tsien*. The unrebutted expert evidence is that a person of skill would indeed be so motivated.

Dr. Metzker provided extensive testimony that a skilled artisan would have been motivated to use the azidomethyl protecting group disclosed in chapter 3 of *Greene & Wuts*. Appx1093 at ¶ 68. This was no hindsight reasoning: Dr. Metzker explained that the chemical group needing protection in *Ju*'s and *Tsien*'s methods, a hydroxyl group ("OH"), can be found in both chapters 2 and 3 of *Greene & Wuts*. Appx1093 at ¶ 68. In fact, *Tsien* itself suggests using azido chemistry as the protecting group.[8] Appx1196. In addition, both *Ju* and *Tsien* recommend small ether groups. Appx1090-1091 at ¶ 60-63. Given this guidance, Dr. Metzker testified that a person of ordinary skill would have been directed to azidomethyl in the index of *Greene & Wuts*. Appx1095 at ¶ 74. Dr. Metzker also explained at length that a skilled artisan would have expected azidomethyl to cleave efficiently. Appx1097 at ¶ 80.

[8] In the related IPR proceeding, the Board in its institution decision (which was undisturbed by the final written decision), found that "*Tsien* discloses using an azido-containing protecting group." Appx170 (citing Appx1011 at ll. 12-17 ("Other blocking modifications to the 3′-OH position of dNTPs include the introduction of groups such as . . . $–N_3$ [*i.e.*, azido] . . . .")).

Illumina argued, and the lower court agreed, that a skilled artisan would have believed that azidomethyl would not be cleaved with the high efficiency required for SBS. The lower court's opinion was based on a view that all the phenol protecting groups are inefficient. *See* Appx11; Appx21 (stating that a skilled artisan would have had to understand that high efficiency was not required before looking to *Greene & Wuts*'s phenol chapter). But in fact *Greene & Wuts* says that "[e]thers are the most widely used protective groups for phenols, and ***in general***, they are more easily cleaved than the analogous ethers of simple alcohols." Appx1494 (emphasis added). But, as Dr. Metzker explained, "Phenolic compounds tend to be a little more reactive than aliphatic alcohols, ***but that's not always true***." Appx1829-1831 (emphasis added). The passage in *Greene & Wuts* does not suggest that a skilled artisan would disregard phenol protecting groups for use with *Ju*'s or *Tsien*'s methods; indeed, as Dr. Metzker explained, the literature suggested azidomethyl could be cleaved with high efficiency. Appx1097 at ¶ 80. In sum, the lower court's conclusion that a skilled artisan would not look to *Greene & Wuts*'s azidomethyl protecting group in developing a method of sequencing cannot stand as a matter of law or fact.

2. The Lower Court Erred by Failing to Take into Account the Creativity of a Person of Ordinary Skill in the Art

The lower court also concluded that a skilled artisan would not have been motivated to use azidomethyl because the cleaving conditions disclosed in

Greene & Wuts would have been too inefficient for use in SBS. Appx10-11. This conclusion is clear error.

The lower court relied on Dr. Metzker's testimony that the cleaving conditions disclosed in *Greene & Wuts* for azidomethyl were not suitable for use in DNA sequencing. Appx10. But the court gave no reason for disregarding Dr. Metzker's further testimony that a skilled person would have understood that he or she was not confined to those cleaving conditions, and that he or she could modify and optimize the conditions to achieve higher efficiency cleavage that could be used for SBS. *See* Appx1099-1100 at ¶ ¶ 83-87 (citing Appx2435-2442; Appx2446-2712). This was a legal error, as the law ***required*** the lower court to consider "the modifications that one skilled in the art would make to a device borrowed from the prior art." *In re Icon Health & Fitness, Inc.*, 96 F.3d 1374, 1382 (Fed. Cir. 2007); *see also Randall Mfg. v. Rea*, 733 F.3d 1355, 1362-63 (Fed. Cir. 2013) (instructing that the background knowledge and creativity of a skilled artisan "must be consulted" in obviousness considerations). This legal error impacted the district court's decision as to the evidence, because the evidence actually showed that a skilled artisan would have been able to choose appropriate cleavage conditions for use of azidomethyl as a protecting group with DNA.

Dr. Metzker not only explained that optimizing cleaving and other chemical reactions is a "basic tenet of organic chemistry," Appx1099 at ¶ 84 citing

Appx2435-2442, but identified two sets of cleavage conditions a skilled artisan would have understood could be used to cleave azidomethyl. Appx1100 at ¶¶ 86-87 (citing Appx2451-2712). On this subject, too, Illumina did not offer evidence in its reply that would rebut Dr. Metzker's testimony.

Moreover, the '537 Patent itself suggests that choosing protecting groups and cleavage conditions for the 3'-OH group was conventional to one of ordinary skill in the art, pointing to Figure 3 of the '537 Patent as merely ***illustrative*** of (***not*** adding to) the common knowledge:

> Suitable protecting groups will be apparent to the skilled person, and can be formed from any suitable protecting group disclosed in Green[e] and Wuts, supra. Some examples of such protecting groups are shown in FIG. 3. The protecting group should be removable (or modifiable) to produce a 3' OH group. ***The process used to obtain the 3' OH group can be any suitable chemical or enzymic reaction.***

Appx35, at 7:65-8:4 (emphasis added). The '537 Patent further demonstrates the routine nature of developing cleaving conditions by not providing any guidance on cleavage conditions for a 3'-OH protecting group, let alone cleavage conditions for an azidomethyl group. *See, e.g., Trustees of Columbia Univ. v. Illumina, Inc.*, 620 Fed. Appx. 916, 933 (Fed. Cir. 2015) ("[I]f novel and nonobvious chemistry was needed to practice the claimed inventions, [the patentee] would have been obligated to disclose this chemistry in the patent."); *In re Kubin*, 561 F.3d 1351, 1356 (Fed. Cir. 2009) (holding that patentee cannot represent in specification that claimed gene sequence can be derived by standard techniques while at the same

time saying that a skilled person would not have been able to use such standard techniques to derive the claimed invention from a prior art reference).

The lower court again erred by failing to considered QIAGEN's unrebutted evidence. If it had, it would have concluded that QIAGEN raised a substantial question as to obviousness.

3. The Lower Court Erred in Dismissing QIAGEN's Labeling Motivation Argument

Apart from a motivation to combine based on sequencing-by-synthesis, QIAGEN introduced evidence below that a skilled artisan interested in DNA labeling methods, whether for step-wise synthesis or for creating a labeled primer, would have looked to the methods of *Ju* and *Tsien*, and that high efficiency cleavage ***would not be required*** for those methods. Appx1094 (citing Appx2271-2363) at ¶¶ 71-73. The lower court did not address this point in the Preliminary Injunction Order, but did discuss the issue in its order granting in part QIAGEN's motion to modify and to stay. Specifically, the lower court faulted Dr. Metzker for "fail[ing] to state that at the time of the '537 patent a skilled artisan would have appreciated th[e] fact [that a method of labeling would not require the high yield or quantitative (*i.e.*, high efficiency) removal], ignored that [high efficiency removal] limitation in *Ju* or *Tsien*, and therefore looked beyond the aliphatic alcohols chapter in *Greene & Wuts* to the chapter on phenols [where azidomethyl is listed as

a removable protecting group]." Appx21. The lower court's reading of Dr. Metzker's testimony simply is mistaken.

Dr. Metzker ***does state*** that a skilled artisan would have appreciated that protecting groups do not need to be cleaved with high efficiency for methods of labeling. *See* Appx1083, Appx1093-1095 at ¶ ¶ 37, 70-74; *see also* Appx1094 at ¶ 71 ("a method of labeling would not require the high yield or quantitative (*i.e.* high efficiency) removal discussed in *Ju* and *Tsien* in the context of using the labeled nucleotide for sequencing"). Dr. Metzker also testified that both *Ju* and *Tsien* included teachings for methods of labeling, and that a person of skill interested in developing such methods would still know of and want to consult SBS references such as *Ju* and *Tsien* for their more general labeling teachings. Appx1093-1094 at ¶ 70; *see also* Appx1090-1091 at ¶ 58, 62 (describing how *Ju* and *Tsien* include teaching methods of labeling). Finally, Dr. Metzker declared that a skilled artisan would have looked to the chapter discussing azidomethyl protecting groups in *Greene & Wuts*, because azidomethyl comported with the labeling-related guidance for protecting groups in *Ju* and *Tsien*: that a group needs to be a small ether that could be incorporated into the nucleic acid strand, which *Greene & Wuts* (and *Tsien*) teach. Appx1093-1095 at ¶¶ 70, 74. Illumina presented no evidence—expert or otherwise—to call into question Dr. Metzker's testimony, and the lower court did not cite any. Ignoring it was clear error.

C. The Lower Court Erred in Concluding That QIAGEN's Alternative Written Description Argument Failed

The lower court agreed with Illumina that a skilled artisan would not have "looked to the 'azido' entry in the index of *Greene & Wuts*," Appx11, but simultaneously rejected QIAGEN's alternative argument that—if the lower court was correct that a skilled artisan would not consult *Greene & Wuts*—claim 6 is invalid for lack of written description. Appx11-12. Illumina cannot have it both ways, and the lower court erred in adopting Illumina's contradictory positions.

Since claim 6, which recites the azidomethyl protecting group, was added during prosecution of the '537 Patent, the azidomethyl limitation must be described in the '537 Patent's specification as originally filed for the claims to be valid. *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323 (Fed. Cir. 2000). However, the '537 Patent specification does not mention azidomethyl. At best, Figure 3 in the '537 Patent discloses a genus of over 2,000 azido protecting groups, only one of which is azidomethyl. However, unless the specification provides "blaze marks" directing an artisan to azidomethyl, claim 6 is invalid for lacking written description. *In re Ruschig*, 379 F.2d 990, 994 (1967) (holding that "the disclosure of a class of 1000, or 100, or even 48 compounds" was not enough to support a claim to a specific compound). As *Ruschig* makes clear, one cannot disclose a forest in the original application, and then later pick a tree out of the forest and say, "Here is my invention." In order to satisfy the written description

requirement, the blaze marks directing the skilled artisan to the inventor's tree must be in the originally filed disclosure. *Purdue Pharma*, 230 F.3d at 1326–27. Here, the only potential "blaze mark" in the '537 Patent is the reference to *Greene & Wuts*, which identifies azidomethyl as an OH protecting group in chapter 3. Appx35, at 7:65-8:4. If the lower court was correct that a skilled artisan would not look to this disclosure, then *Greene & Wuts* is no blaze mark at all, and claim 6 lacks adequate written description.

In addressing QIAGEN's argument, the lower court attempted to reconcile its discordant conclusions by pointing to the general disclosure of azido in the specification of the '537 Patent:

> Although there is no support for the assertion that a skilled artisan would have looked to the "azido" entry in the index of *Greene & Wuts* before the '537 patent, the '537 patent itself, by focusing on azido groups as protecting groups that could be removed leaving an OH-group exposed, would likely have provided the blaze marks that were otherwise absent from the prior art.

Appx12.[9] However, the court's explanation does not hold up to scrutiny because claim 6 (as opposed to the other asserted claims), is directed to an azidomethyl group, not generic "azido groups."

[9] The lower court also concluded that "[i]n any case, it would only invalidate claim 6, which is just one of seven claims asserted herein, so Qiagen could not escape liability by succeeding on its written description argument." Appx14. However,

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If this Court finds that the lower court did not err in concluding that a person of skill in the art would not look to the azidomethyl teaching in *Greene & Wuts*, claim 6 must be invalid for lack of written description and the Court should reverse the lower court as to that claim.

II. THE LOWER COURT'S FINDING OF IMMINENT, IRREPARABLE HARM WAS NOT SUPPORTED BY ANY EVIDENCE AND, THEREFORE, WAS CLEARLY ERRONEOUS

The lower court's finding of irreparable harm was based on findings that were adopted wholesale from the conclusory, speculative declaration of an Illumina executive, and unsupported by expert or documentary evidence. Appx16 (citing Appx1055-1057; Appx1060-1061; Appx1069 at ¶¶ 13-18, 26-29, 45). This Court has cautioned against granting injunctions based on a plaintiff's conclusory assertions of harm. *See Atari Games Corp. v. Nintendo of Am., Inc.*, 897 F.2d 1572, 1575 (Fed. Cir. 1990).

Speculation as to loss of market share, or the "mere showing that [the patentee] might lose some insubstantial market share as a result of [the defendant's] infringement is not enough" to entitle a patent owner to a preliminary injunction. *Apple Inc. v. Samsung Elecs. Co.*, 678 F.3d 1314, 1324-25 (Fed. Cir. 2012). "[L]ost market share must be proven (or at least substantiated with some

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QIAGEN also argued that claims 1-5, and 8 are invalid for failing to enable the full scope of the claimed azido groups. Appx15.

evidence) in order for it to support entry of a preliminary injunction." *Automated Merch Sys. v. Crane Co.*, 357 Fed. Appx. 297, 301 (Fed. Cir. 2009); *see also Nutrition 21 v. United States*, 930 F.2d 867, 871 (Fed. Cir. 1991) (reversing preliminary injunction that was based on speculative evidence of "possible market share loss"). In order to be entitled to an injunction, Illumina was required to supply, and the lower court was required to base its decision on, ***evidence*** that Illumina faces "a likelihood of ***substantial and immediate*** irreparable injury." *Apple, Inc.*, 678 F.3d at 1325. Nothing in the evidence supports the conclusion that GeneReader is likely to take "substantial" market share from Illumina at any time prior to trial on the merits in this case, much less imminently.

The conclusions by the lower court concerning the market and the supposed threat of irreparable harm to Illumina were clear error based on the evidence before it. The lower court concluded that QIAGEN threatened to "capture and redefine" the market for benchtop DNA sequencers, which the court said was at a "crucial inflection point in the development of the market for DNA sequencing equipment for clinical laboratories." Appx17 (citing *Celsis In Vitro, Inc. v. CellzDirect, Inc.*, 664 F.3d 922 (Fed. Cir. 2012)); *see also* Appx16 ("market for DNA sequencing in clinical laboratories is expected to grow substantially in the near future").

The lower court did not cite any evidence to support its characterization of the clinical laboratory market as at an "inflection point," nor attempt to reconcile its (and Illumina's) description of the market as somehow "new," with Illumina's own significant sales in that market for the last several years. *See* Appx1632; Appx1627 (Illumina controlled [redacted] of the clinical laboratory NGS market in 2014); Appx1577-1578.

The lower court's misreading of the evidence is demonstrated by its treatment of a portion of deposition testimony from QIAGEN declarant Jonathan Arnold for the proposition that "Qiagen's own assessment of its prospects project it ***will become a significant player in the coming year***." Appx17 (citing Appx1744; emphasis added). But Mr. Arnold made clear that QIAGEN's projections for GeneReader were limited to a "very narrow market segment," Appx1855-1856, and that QIAGEN views GeneReader as competitive with Thermo Fisher's benchtop sequencer, not Illumina's products. Appx1864-1866.

This Court's decision in *Celsis* on which the lower court relied highlights the lower court's clear error in describing the state of the market in which QIAGEN seeks to compete with Illumina. In *Celsis*, the district court heard unrebutted testimony from plaintiff's expert on damage to plaintiff's pricing, reputation, and business opportunities. The plaintiff in *Celsis* also proved that it had to break a "no-discount" policy that it previously enforced to establish

premium product pricing, and that the market "was particularly sensitive because customers buy in bulk and at irregular times, such that the loss of a single sale in this market may be more harmful than for products purchased daily." *Celsis In Vitro, Inc.*, 664 F.3d at 930.

Illumina did not present ***any*** evidence that the market for NGS sequencing instruments is at an "inflection point," that it suffered the harms inflicted on plaintiff in *Celsis*, or that it imminently will. Plainly stated, clinical laboratories purchasing NGS technology is not some "new" market in which Illumina and QIAGEN are the first two entrants. Illumina, Thermo Fisher, and others have been selling benchtop sequencers to these customers for years, and QIAGEN now is attempting to compete with those companies (and others Illumina identifies as developing NGS products) with GeneReader.[10] Illumina did not

[10] The lower court did not address that there are other competitors in this market, including one, Thermo Fisher, that (1) already is Illumina's second-largest competitor; (2) offers a benchtop sequencer for a lower price than GeneReader and all but one of Illumina's sequencers; (3) offers alternative pricing models to customers; and (4) has the same dedicated sales force and relationships with clinical laboratories that Illumina claims make QIAGEN such a threat. Appx1589-1590; Appx1121, at ¶ 28. In addition, Thermo Fisher's benchtop sequencer is more "on par" to GeneReader, Appx1866, and therefore could be just as responsible for Illumina's hypothesized market impact as QIAGEN's GeneReader. The lower court's failure to even acknowledge this competition in concluding that Illumina demonstrated QIAGEN's GeneReader is a substantial threat was error.

submit any evidence to the contrary, and the lower court erred in basing its irreparable harm finding on Illumina's unsupported claims.

The lower court's conclusion that QIAGEN would take significant market share from Illumina because of QIAGEN's "disruptive" pricing also was clear error. To the contrary, the unrebutted evidence was that, even with these pricing options, QIAGEN had sold only [redacted] GeneReaders in the U.S. during the ten months it was on the market before the preliminary injunction. Appx1118 at ¶ 7. Moreover, the lower court ignored Illumina's admission that these alternative pricing models had been around for years, and indeed were offered by Illumina and other competitors. Appx1588-1591. Finally, notwithstanding QIAGEN's pricing models, Illumina had not made any changes to its pricing, or even decided that it would change in the future. Appx1614-1615. These points are crucial, as a pricing-related harm must be supported by evidence that the harm is both likely to happen and ***not compensable by damages***—such as when a low cost knockoff would drive the patentee from the market altogether. *Automated Merchandising Systems*, 357 Fed. Appx. at 301. There was no such evidence here, and the undisputed evidence is that both Illumina and QIAGEN has detailed information regarding the revenues generated by the sale of sequencing instruments, and the

follow-on sales of consumable chemical reagents customers use when sequencing DNA.[11] Appx1574-1576; Appx1120 at ¶ ¶ 21-22.

Finally, the lower court concluded—again without evidentiary support—that GeneReader might perform "poorly," and cause irreparable harm to Illumina by sullying the reputation of NGS and souring the clinical laboratory market on the technology. Appx17. There is zero evidence to support the lower court's hypothesized impact; the only evidence was to the contrary. First, the lower court's conclusion that GeneReader may perform "poorly" relies on Illumina's mischaracterization of testimony by QIAGEN's Senior Director, Mr. Arnold.

Mr. Arnold explained that, because the GeneReader sequencing performance is not as robust as Illumina's benchtop sequencers, QIAGEN made GeneReader a more limited, "closed" system that currently only sequences certain cancer genes in QIAGEN's cancer panel. Mr. Arnold noted that, in contrast,

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[11] Illumina, in a single conclusory paragraph in one of its supporting declarations, asserted that the possibility for "irreversible discounting" as a result of QIAGEN's alternative pricing was a source of irreparable harm. *See* Appx1069, at ¶ 45. The lower court did not rely on this theory of harm in the Preliminary Injunction Order, instead finding that QIAGEN allowing customers to purchase GeneReader on a conditional basis, which includes a trial period in which customers could validate GeneReader's performance, supposedly would substantially impact sales and market share. Appx16-17. There is no evidence to support either theory.

Illumina's "open" sequencers can sequence biological matter for whatever a lab wishes. Appx2164; Appx2168-2170. Thus, the point was that GeneReader works in a more limited manner than Illumina's benchtop sequencing products, ***not*** that GeneReader does not work at all, or does not perform the functions for which it is marketed. *See* Appx2162-2165; Appx2168-2169 (explaining development of GeneReader as a "closed" system that allows for sequencing of certain genes).

More important, even if GeneReader performed "poorly," there is no evidence at all to support the lower court's speculation about the impact of "poor" performance on the market. The only passage from Mr. Arnold's deposition the court cited to support its conclusion that a customer frustrated with GeneReader would abandon NGS technology entirely said precisely the opposite. Under cross-examination on this issue by Illumina, Mr. Arnold repeatedly said, "no," when Illumina's counsel argued this hypothetical impact with him. *See* Appx1861-1862.

Despite GeneReader being on the market for over ten months by the time the lower court issued the Preliminary Injunction Order, Illumina presented no evidence of even a single lost sale, any loss of its market share, or any decline in the prices of its benchtop sequencers. Appx1616. The evidence from Illumina's own documents and witness was that neither Illumina nor QIAGEN expected the GeneReader to "capture" a substantial portion of the sequencer market in the near term, "redefine" the market by eroding prices for sequencers, or do anything to

harm Illumina's reputation or the market for NGS in clinical laboratories. Illumina is the dominant player in the market for DNA sequencing instruments and has been for years, consistently owning the lion's share of the market, and publicly touting that Illumina machines produce 90% of the world's sequencing data. In contrast, QIAGEN holds less than [redacted] of the NGS market (almost all of which is attributable to the peripheral devices and consumable reagents QIAGEN sells).

The lower court's contrary conclusion was clearly erroneous, and the Preliminary Injunction Order should be reversed because Illumina did not satisfy its burden to prove irreparable injury.

CONCLUSION

For all the foregoing reasons, QIAGEN requests that the Court reverse the lower court's grant of a preliminary injunction in this case, and remand the case to the lower court for further proceedings on Illumina's infringement claims.

Dated: October 14, 2016

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ADDENDUM

IN THE UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF CALIFORNIA

ILLUMINA, INC., and ILLUMINA CAMBRIDGE LTD.,

Plaintiffs,

v.

QIAGEN, N.V., QIAGEN GmbH, QIAGEN GAITHERSBURG, INC., QIAGEN SCIENCES, LLC, QIAGEN INC. (USA), QIAGEN REDWOOD CITY, INC., and INTELLIGENT BIO-SYSTEMS, INC.,

Defendants.
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No. C 16-02788 WHA

ORDER GRANTING MOTION FOR PRELIMINARY INJUNCTION

INTRODUCTION

In this patent infringement action involving DNA sequencing technology, the patent owner moves for a preliminary injunction. For the reasons stated below, the motion for a preliminary injunction is **GRANTED**.

STATEMENT

Plaintiff Illumina Cambridge, Inc., owns U.S. Patent No. 7,566,537, which covers "Labelled Nucleotides." Plaintiff Illumina, Inc., is the exclusive licensee of the '537 patent. Illumina and Illumina Cambridge (collectively, "Illumina") sell DNA sequencing equipment that practices the '537 patent (Van Oene Decl. ¶ 6).

Defendant Qiagen N.V. and several of its subsidiaries, defendants Qiagen GmbH, Qiagen Gaithersburg, Inc., Qiagen Sciences, LLC, Qiagen Inc. (USA), Qiagen Redwood City, Inc., and Intelligent Bio-Systems, Inc. (collectively, "Qiagen"), jointly developed and

announced the launch of a competing product, the GeneReader NGS System, with plans to begin distribution later this year. Illumina now seeks to enjoin sales of Qiagen's GeneReader products.

Before discussing the details of the technology at issue herein, some background on the science of DNA is necessary.

1. DNA.

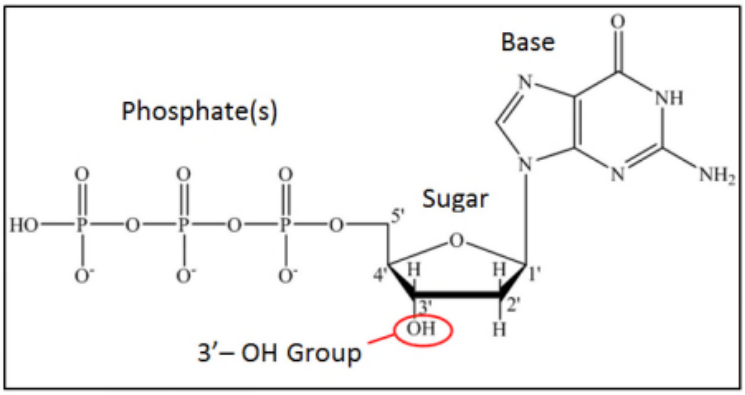
DNA, which stands for deoxyribonucleic acid, encodes the genetic material of most organisms. DNA comprises a double helix of strands of linked molecules called nucleotides. Each nucleotide contains a sugar, a phosphate, and one of four different chemical bases, adenine ("A"), cytosine ("C"), guanine ("G"), and thymine ("T"). The bases pair with each other — A with T and C with G — and the two strands of the double helix are held together by the bonds between complementary bases. In other words, each side of a DNA double helix is a perfect complement of the other. The sequence of the bases in a DNA strand reflects genetic information (Metzker Decl. ¶¶ 28–31).

As stated, each nucleotide contains a sugar chemical group, which is a ring comprising five carbon atoms. By convention, the carbon atoms are numbered one prime (1') through five prime (5'), and each such atom can be bound to another atom or chemical group, depending on the specific kind of sugar used in that nucleotide. The nucleotides in DNA use the sugar deoxyribose. Deoxyribose has a hydroxyl group (one hydrogen atom and one oxygen atom) at the 3' position (known as a "3'-OH group"). Deoxyribose has only a hydrogen atom at its 2' position. The name deoxyribose indicates the lack of an oxygen atom at the 2' group as compared to ribose, a sugar used in a different kind of genetic material known as RNA, which has a hydroxyl at the 2' position (as well as at the 3' position).

The phosphate group of a nucleotide is attached at the 5' position of the sugar. As stated, the strands of DNA comprise a series of nucleotides. The nucleotides in the series are connected via bonds between the 3'-OH group of the sugar on one nucleotide and the phosphate group of the next nucleotide. This sugar-phosphate bond forms the backbone of each strand of

a DNA double helix, and the pairwise bonds between the bases (A-T and C-G) form the cross-bars of the helix.

Below is an image depicting a single nucleotide featuring deoxyribose (Metzker Decl., Fig. 1):



G Nucleotide

The chemical group labeled "Base" is a G (guanine) group. The vertices of the sugar ring (labeled 1' through 4') and the additional point extending from the 4' position (labeled 5'), are carbon atoms. They are not labeled with a "C" by convention. The circled 3'-OH group is, as stated, the connecting point between each nucleotide and the phosphate group of the next. It is a key aspect of the technology herein, to which this order now turns.

2. SEQUENCING-BY-SYNTHESIS.

Because DNA contains two perfectly complementary strands of nucleotides, the full sequence of DNA can be determined by identifying the sequence of bases in the nucleotides on one of the strands and inferring (from the A-T/C-G pairing) the sequence of the other strand. One technique, used by both Illumina and Qiagen, for identifying sequence of A, T, C, and G that make up one strand is called "sequencing by synthesis." This process first involves unwinding the double helix of the DNA sought to be analyzed, retaining one strand as a template, and affixing that template to a surface to maintain stability throughout the sequencing process. Once the template is affixed, an enzyme proceeds along the template, adding complementary nucleotides to an adjacent "primer" strand, which is positioned to be read by the

sequencing device. The enzyme proceeds stepwise until it has added a complementary nucleotide for the entire chain.

The nucleotides added in the sequencing-by-synthesis process differ from the natural nucleotides in two critical ways. *First*, each nucleotide is modified to include a chemical label, unique to each base (A, T, C, or G) and attached to that base, that can be detected by an external device, such as by emitting a unique fluorescent display. *Second*, each nucleotide is modified to include a "blocking group" or "protecting group" (the terms are used interchangeably) that prevents further nucleotides from binding with the 3'-OH group of the sugar. This forces the enzyme to pause and wait until the label for a nucleotide has been detected before adding the next nucleotide.

Once the nucleotide label has been detected, the label can be removed (to avoid interference with further detection), and, critically for our case, the protecting group can be removed in a manner that leaves the 3'-OH group of the sugar exposed, allowing another nucleotide to be added.

The '537 patent claims a method for labeling nucleotides in this manner and specifically using an "azido group" as the protecting group. (The patent does not specifically require *sequencing*.) Specifically, Claim 1 of the patent, the only independent claim asserted herein, reads as follows:

> A method of labeling a nucleic acid molecule, the method comprising incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule, wherein the nucleotide or nucleoside molecule has a base that is linked to a detectable label via a cleavable linker and the nucleotide or nucleoside molecule has a ribose or deoxyribose sugar moiety, wherein the ribose or deoxyribose sugar moiety comprises a protecting group attached via the 2' or 3' oxygen atom, and said protecting group can be modified or removed to expose a 3' OH group and the protecting group comprises an azido group.

An azido group is a chemical group including three nitrogen atoms (N_3), *inter alia*. The set of OH protecting groups comprising an azido group encompasses a broad class of more than one thousand chemical structures (Metzker Decl. ¶ 100). Claim 6 of the patent, which depends from Claim 1, is limited to a single protecting group known as azidomethyl.

The patent defines the term "nucleoside" as follows ('537 patent, col. 4, l. 59–63):

> A "nucleoside" is structurally similar to a nucleotide, but are [sic] missing the phosphate moieties. An example of a nucleoside analog would be one in which the label is linked to the base and there is no phosphate group attached to the sugar molecule.

In other words, a nucleoside is a chemical group that, like a nucleotide, includes a sugar and a base, but *unlike* a nucleotide, lacks a phosphate group. Nucleosides are discussed in greater detail in connection with Qiagen's enablement argument below.

3. QIAGEN'S GENEREADER.

Since Intelligent Bio-Systems became a part of the Qiagen family of companies in 2012, Qiagen repeatedly teased the announcement of DNA sequencing technology, then delayed the launch of the product. In April 2016, however, Qiagen began aggressively marketing its GeneReader NGS system. Qiagen itself stated in its marketing materials that GeneReader worked in the "same way as Illumina's machines, flooding the sample DNA with flourescently labeled nucleotides and imaging the results" (Walter Decl., Exh. 1 at 2). Indeed, Qiagen's manual described a process very similar to the patented invention (*id.*, Exh. 13 at 20). Qiagen's devices used nucleotides including labels linked to their base via a cleavable linker, and they all used protecting groups that comprised azido groups (Burgess Decl., Appx. 1 at 5–13).

Although Illumina established a strong brand in the market for DNA sequencing products, Qiagen's GeneReader began to compete with several of Illumina's sequencing products, specifically in targeting clinical laboratories, where affordable desktop sequencing devices had just taken off. Qiagen offered its product based on a novel pricing structure, whereby customers paid per use, rather than purchasing the machine and other ancillary products outright (Van Oene Decl. ¶¶ 13–18, 22–26, 45)

4. PROCEDURAL HISTORY.

In 2012, Intelligent Bio-Systems and non-party the Trustees of Columbia University in the City of New York sued Illumina for patent infringement in the District of Delaware. Illumina asserted counterclaims of infringement of the '537 patent (pertaining to predecessors of the GeneReader). After Qiagen N.V. acquired Intelligent Bio-Systems, Illumina added Qiagen N.V. as a new party in that action and asserted counterclaims against it as well. Qiagen

N.V. moved to dismiss for lack of personal jurisdiction, and Illumina voluntarily dismissed all claims against it without prejudice. Illumina maintained its claims against Intelligent Bio-Systems and took some discovery relating to the accused products herein in that case.

In 2013, Intelligent Bio-Systems challenged the '537 patent on obviousness grounds in an *inter partes* review proceeding before the Patent Trial and Appeals Board. (The Delaware action was stayed pending that review.) The PTAB instituted review of the patent based on several of the prior art references raised in the petition, but declined to institute review as to others, finding them redundant. The PTAB upheld the validity of the claim, and the Federal Circuit affirmed the decision. *See Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359 (Fed. Cir. 2016).

Just two weeks after the Federal Circuit's decision upholding the validity of the '537 patent in May 2016, Illumina commenced this action. It filed the instant motion for a preliminary injunction the same day. While the motion remained pending, defendants moved to transfer the action to the District of Delaware, in deference to an earlier-filed action involving the same patent and defendant Intelligent Bio-Systems. Qiagen N.V. also moved to dismiss for lack of personal jurisdiction. The parties stipulated to a schedule by which the motions to transfer and dismiss would be resolved before the preliminary injunction (Dkt. Nos. 34–35).

An order denied the motion to transfer, reserving on a final decision after the conclusion of jurisdictional discovery relating to Qiagen N.V.'s contacts with California as well as with the United States as a whole (Dkt. No. 64). Rather than bear the burden of jurisdictional discovery, Qiagen N.V. consented to personal jurisdiction in this district, and its motion to dismiss was denied as moot (Dkt. Nos. 82–83). This order addresses Illumina's motion for a preliminary injunction. It follows full briefing and oral argument.[1]

[1] Qiagen seeks leave to file a surreply to address new arguments and evidence concerning irreparable harm and invalidity raised for the first time in Illumina's reply. Qiagen's proposed surreply responds to several arguments raised for the first time in Illumina's reply, but the brief also raises several new arguments not raised in the opposition. Additionally, Illumina objects to Qiagen's proposed surreply, stating the parties agreed to a briefing schedule pursuant to which Qiagen would not seek to file post-reply submissions unless Illumina filed declarations and Qiagen took depositions of the declarants. The stipulation referenced does not include such a limitation, it merely provides such depositions as an option (Dkt. No. 35 at ¶ 4(a)). In its objection, Illumina identifies several new arguments in Qiagen's surreply.

ANALYSIS

"A preliminary injunction is a 'drastic and extraordinary remedy that is not to be routinely granted.'" *National Steel Car, Ltd. v. Canadian P. Ry., Ltd.*, 357 F.3d 1319, 1324 (Fed. Cir. 2004) (quoting *Intel Corp. v. ULSI Sys. Tech., Inc.*, 995 F.2d 1566, 1568 (Fed. Cir. 1993)). A party seeking a preliminary injunction must show: "(1) it is likely to succeed on the merits; (2) it is likely to suffer irreparable harm if the injunction is not granted; (3) the balance of hardships weighs in its favor; and (4) an injunction is in the public interest." *Apple v. Samsung* ("*Apple II*"), 695 F.3d 1370, 1373–74 (Fed. Cir. 2012). This order addresses each factor in turn.

1. LIKELIHOOD OF SUCCESS ON THE MERITS.

Illumina accuses Qiagen of infringing independent Claim 1 and dependent Claims 2–6 and 8 with its GeneReader product. Its technical expert, Professor Kevin Burgess, provided a detailed declaration setting forth how each element of each of the asserted claims appears in the GeneReader (Burgess Decl. ¶¶ 49–87). Qiagen does not deny that Illumina is likely to succeed on the merits of showing that the accused products read on each of the asserted claims of the '537 patent. Instead, Qiagen contends that the patent is invalid.

At trial, Qiagen will bear the burden of overcoming the statutory presumption of validity with clear and convincing evidence. At this stage, however, we need "not resolve the validity question, but rather must . . . make an assessment of the persuasiveness of [Qiagen's evidence]" recognizing that further evidence favoring either side may be presented at trial. *Titan Tire Corp. v. Case New Holland, Inc.*, 566 F.3d 1372, 1377 (Fed. Cir. 2008).

The Federal Circuit directs us to "first weigh the evidence both for and against validity" as available at the preliminary injunction stage, then to assess whether there is a "substantial question" concerning the validity of the patent, "meaning that the alleged infringer has presented an invalidity defense that the patentee has not shown lacks substantial merit . . . ." *Id.* at 1379. Likelihood of success on the merits is a probability of fifty-one percent or more. That

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The parties' disregard for the procedures of this Court is disappointing. Nevertheless, this order considers the arguments in Qiagen's surreply and unauthorized supplemental letter and finds them unavailing.

leaves a forty-nine percent likelihood that the accused infringer would succeed, which leaves more than enough room for a "substantial question." Thus, this focus on the presence (or absence) of a "substantial question" of validity is at odds with the primary inquiry at this stage, which considers *likelihood* of success on the merits.

The decision in *Titan Tire* recognized that tension and sought to clarify the appropriate standard. It reiterated the foregoing discussion regarding a substantial question of invalidity, but determined as follows:

> It is important to remember that, however engaged the court may be in the process of determining whether the alleged infringer has shown a "substantial question" of invalidity as we have explained it, that process does not change the court's ultimate decision point . . . has the plaintiff established a likelihood of success on the merits? Asking whether the challenger has raised a substantial question of invalidity in the manner we have described may be a useful way of initially evaluating the evidence, but the ultimate question regarding the first preliminary injunction factor remains that of the patentee's likelihood of success on the merits.

Ibid. Thus, this order considers whether, in light of the evidence presented by both sides at this stage, Illumina has shown it is *likely* to defeat Qiagen's invalidity arguments, for which Qiagen will ultimately face the clear and convincing evidence standard. Qiagen argues that the '537 patent is invalid inasmuch as it is obvious in light of the combination of three references in the prior art. It also argues that the '537 fails to enable a person of ordinary skill in the art to practice the full scope of the claimed invention.

A. Obviousness.

Qiagen argues that the '537 patent is obvious in light of three prior art references. The three articles of prior art are: (1) Roger Tsien, *et al.*, WO 91/06678 (May 16, 1991) ("Tsien"), (2) Jingyue Jue, *et al.*, U.S. Patent No. 6,664,079 (Dec. 16, 2003) ("Ju"), and (3) Theodora W. Greene & Peter G.M. Wuts, Protective groups in Organic Syntheis 246–92 (3d ed. 1999) ("Greene & Wuts"). To establish that a patent claim is obvious, a challenger must show that all of the claimed elements were known in the prior art and that a skilled artisan "would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so."

Kinetic Concepts, Inc. v. Smith & Nephew, Inc., 688 F.3d 1342, 1360 (Fed. Cir. 2012) (citations omitted).

Both Tsien and Ju described modified nucleotides, such as those claimed in the '537 patent, that comprised a base linked to a detectable label that could be removed, a protecting group at the 3' position, and the ability to remove the protecting group to expose a 3'-OH group, though neither Tsien nor Ju identified azido groups as viable removable protecting groups. Additionally, Tsien and Ju utilized a procedure for removing a protecting group that required a high level of efficiency — a limitation not present in the '537 patent.

Qiagen's obviousness argument turns on whether a skilled artisan would have found it obvious to combine the labeled nucleotides taught by Tsien and Ju with any references to azido groups in Greene & Wuts.

As a preliminary matter, Illumina argues that each of our defendants is estopped from asserting the obviousness arguments raised herein, because defendant Intelligent Bio-Systems already unsuccessfully challenged the '537 patent through the *inter partes* review procedure before the PTAB and on appeal at the Federal Circuit. Section 315(e)(2) of Title 35 of the United States Code provides that the petitioner in an IPR proceeding, "or the real party in interest or privy of the petitioner, may not assert . . . in a civil action . . . that the claim is invalid on any ground that the petitioner raised or reasonably could have raised during that inter partes review."

Intelligent Bio-Systems identified Greene & Wuts (combined with Ju or Tsien) in its petition, but the PTAB did not institute IPR proceedings as to those references. It instituted the proceedings as to Ju or Tsien in combination with Zavgorodny, and concluded that consideration of Greene & Wuts would have been redundant.

The Federal Circuit recently held that statutory estoppel does not apply to grounds raised in a petition but not instituted. *Shaw Industries Group, Inc. v. Automated Creel Systems, Inc.*, 817 F.3d 1293, 1300 (Fed. Cir. 2016). Thus, the arguments that Qiagen raises herein, which were not instituted by the IPR, are not barred by Section 315(e)(2). Illumina also offers

no authority supporting its contention that *all* defendants herein are "privies" of Intelligent Bio-Systems, simply because they are all ultimate subsidiaries of the same parent.

For the first time in its reply, Illumina argues that common law issue preclusion, rather than the preclusion rules set forth in Section 315(e)(2), prevent Qiagen from advancing this argument. Illumina cites *B&B Hardware, Inc. v. Hargis Industrial*, 575 U.S. ___, 135 S.Ct. 1293, 1305 (2015), for the proposition that common law preclusion applies here, but that decision concerned review of a decision at the Trademark Trial and Appeal Board under the Lanham Act, which does not set forth explicit terms for estoppel. *B&B Hardware* specifically noted, "courts may take it as a given that Congress has legislated with the expectation that the principle [of issue preclusion] will apply except when a statutory purpose to the contrary is evident." *Ibid.* (citing *Astoria Fed. Sav. and Loan Ass'n v. Solimino*, 501 U.S. 104 (1991)) (alteration in original).

Unlike the Lanham Act, Section 315(e)(2) set forth the bounds of estoppel based on IPR proceedings. Although this order need not conclusively resolve the issue of estoppel at this stage, Illumina is unlikely to prevail in displacing the statutory design of Section 315(e)(2) in favor of the common law. Nevertheless, this order finds Qiagen's obviousness argument unpersuasive.

Greene & Wuts is an extensive treatise covering thousands of protecting groups for various purposes including for hydroxyl groups, such as those at the 3' position of deoxyribose. Greene & Wuts does teach the use of azidomethyl (the specific azido claimed in Claim 6 of the '537 patent), as a protecting group, but that reference is in a chapter directed at phenols, which are hydroxyl groups but of a different type than the hydroxyl group that appears in nucleotides or nucleosides (Liaw Decl., Exh. 4, Greene & Wuts at 260). Greene & Wuts offers an entirely separate chapter on aliphatic alcohols, which include the types of hydroxyl groups that appear in nucleotides and nucleosides. That chapter makes no mention of azido groups. Further, Qiagen's own expert acknowledges that Greene & Wuts teaches removal of the azidomethyl group using a compound that would be inappropriate for use with a nucleotide because "it would alter DNA structures" (Metzker Dep. at 161).

Qiagen and its expert contend that, rather than looking to the chapter specifically addressing aliphatic alcohols, a skilled artisan would have looked for "azido" in the index of Greene & Wuts to find an appropriate protecting group. Qiagen's expert herein, Dr. Michael Metzker, asserted that a skilled artisan would have been so motivated because Tsien taught using azido groups to protect a 3'-OH group (Metzker Decl. ¶ 64). But during the IPR proceedings, Qiagen's expert stated the azido group referenced in Tsien would not have been removable to expose a 3'-OH group (Branchaud Dep. at 101). At his deposition, Dr. Metzker affirmed that Tsien's reference to azido groups concerned the use of such groups directly connected to the carbon atom at the 3' position, which, if removed, would not expose a 3'-OH group (Metzker Dep. at 102–03).

At this stage, Qiagen has made a weak showing that a person of skill in the art would have been motivated to look to the reference to azidomethyl in the phenol chapter of Greene & Wuts to find an appropriate blocking group for the methods described in Tsien or Ju, which addressed aliphatic alcohols, not phenols.

Moreover, Greene & Wuts taught that use of the category of compounds that includes azidomethyl ("ethers") would have been more efficient for use in phenol than in aliphatic alcohols (such as the 3'-OH in a nucleotide) (Liaw Decl., Exh. 4, Greene & Wuts at 248). Thus, even if Tsien or Ju provided adequate motivation to a skilled artisan to have consulted that chapter of Greene & Wuts, that reference would have indicated a low likelihood of success in using azidomethyl in the process taught by Tsien and Ju, which required high-efficiency removal of the 3'-OH protecting group.[2]

In its surreply, Qiagen notes that Claim 6, which specifically claims the use of an azidomethyl as the azido group of Claim 1, was added during the prosecution of the '537 patent. Thus, information adequate to support that claim must have been disclosed in the application *as*

[2] Although it did not institute the IPR proceeding as to Green & Wuts, the PTAB acknowledged this aspect of Greene & Wuts as a background reference that supported its rejection of Intelligent Bio-Systems' arguments at the IPR proceeding, and the Federal Circuit affirmed that this reference in Greene & Wuts "support[ed] a conclusion that the claimed efficiency that allegedly motivated the combination would not be achieved and that a person of ordinary skill in this field would not have been motivated to use" azidomethyl. *Intelligent Bio-Systems*, 821 F.3d at 1368–69.

filed. *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323 (Fed. Cir. 2000). The '537 patent pointed to Greene & Wuts as a resource for a suitable protecting group, but it did not specifically call out azidomethyl or phenol alcohols. Thus, Qiagen argues that if a skilled artisan would not have looked to the chapter on phenol alcohols and the section about azidomethyl before the '537 patent, reference to Greene & Wuts generally was an inadequate disclosure of azidomethyl in the patent itself. "One cannot disclose a forest in the original application and then later pick a tree out of the forest and say here is my invention." *Id.* at 1326–27 (Fed. Cir. 2000). Rather, there must be "blaze marks" directing a person of skill in the art to that particular "tree." *Id.* at 1327.

Although there is no support for the assertion that a skilled artisan would have looked to the "azido" entry in the index of Greene & Wuts before the '537 patent, the '537 patent itself, by focusing on azido groups as protecting groups that could be removed leaving an OH-group exposed, would likely have provided the blaze marks that were otherwise absent from the prior art. Because Qiagen raised this argument for the first time in its surreply, Illumina did not have the opportunity to present argument or evidence in response, but this order finds Qiagen's written description argument unpersuasive. In any case, it would only invalidate Claim 6, which is just one of seven claims asserted herein, so Qiagen could not escape liability by succeeding on its written description argument.

At this stage, Illumina appears likely to overcome Qiagen's obviousness challenge (and the related written description challenge) particularly in light of the presumption of invalidity and the clear and convincing evidence standard.

B. Enablement.

Section 112(a) of Title 35 of the United States Code provides:

> The specification [of a patent] shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same . . . .

After arguing that the combination of every element of the inventions claimed by the '537 patent would have been obvious to a skilled artisan based on the prior art, Qiagen reverses

field and argues that it would not have been obvious from the specification and prior art how to practice the inventions, that is, the '537 patent itself did not enable a skilled artisan to practice the inventions. Specifically, Qiagen contends that the '537 patent suffered from three enablement deficiencies: (i) it did not teach how to use nucleosides, rather than nucleotides, (ii) it did not teach how to use the full scope of azido protecting groups, and (iii) it did not teach how to use a nucleotide with an azido group attached at the 2' oxygen atom in a nucleotide containing a ribose (or other sugar comprising an oxygen atom at that position). Each is addressed in turn.

(i) Nucleosides.

Qiagen argues that the '537 patent did not adequately teach how to use a nucleoside molecule. Claim 1 recited a method comprising "incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule . . . ." As stated, a nucleoside is similar to a nucleotide, except it lacks a phosphate group — the group that binds a nucleotide molecule to the 3'-OH group on the previous nucleotide. Qiagen contends that the '537 patent failed to disclose how a nucleoside could be incorporated into a nucleic acid, as the patent claimed.

Experts on both sides acknowledge that a nucleotide might alternatively be referred to as a "nucleoside triphosphate," indicating that the nucleotide included a nucleoside plus a phosphate group (Romesberg Dep. at 26; Metzker Dep. at 76). Illumina further notes that numerous patents and applications claim the incorporation of a "nucleotide or nucleoside" into a nucleic acid, *including an application by Qiagen's expert in this very case* (*see* Walter Decl., Exh. 9 at 36, 189, Exh. 11). On the other hand, the specification expressly defined a nucleoside as "structurally similar to a nucleotide, but . . . missing the phosphate moieties" ('537 patent, col. 4, l. 59–60).

Illumina argues that the patent should be read to reflect the state of the art, namely, that a skilled artisan might alternatively have referred to a nucleotide as a nucleoside moiety combined with a phosphate group. Thus, Illumina argues, because the '537 patent included the claim limitation "nucleotides or nucleosides," rather than just "nucleotides," one could not evade a finding of literal infringement by describing the nucleotides in use as nucleoside

triphosphates (or some other nucleosides combined with phosphate groups). Qiagen's only evidence to the contrary is the lame opinion of its expert that even his own patent application was invalid because it claimed the incorporation of nucleotides or nucleosides into a nucleic acid.[3]

Even if Qiagen could ultimately prevail on this point, it would face an additional hurdle, inasmuch as Claim 4 of the '537 patent (also asserted herein) claims "[t]he method of Claim 1, wherein the nucleotide is a deoxyribonucleotide triphosphate," thereby specifying the use of a nucleotide, rather than a nucleoside. At oral argument and in an unauthorized letter brief after the hearing, Qiagen argued that Section 112(d) of Title 32 of the United States Code provides, "[a] claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers." Thus, Qiagen contends that Claim 4 should be read to cover the incorporation of deoxyribonucleotide triphosphate *or* any nucleosides into a nucleic acid.

Section 112(d) requires us to construe a dependent claim as *narrowing* the claim from which it depends. Qiagen contends it requires us to rigidly construe a dependent claim as narrowing antecedent claim only to a point and no further, but Qiagen cites no authority for its position. On the contrary, a skilled artisan would have been more likely to understand Claim 4 as electing to use a nucleotide among the choice between nucleotides or nucleosides, and *further* electing to use deoxyribonucleotide triphosphate. Thus, even if Qiagen can show that Illumina failed to enable use of nucleosides, it likely could not escape liability for infringing Claim 4.

This order finds that Illumina is likely to succeed in proving that a skilled artisan would have understood the reference to nucleosides in the patent claims to include the nucleosides that, when ultimately combined with a phosphate group, become nucleotides. Qiagen is unlikely to show clear and convincing evidence to the contrary.

[3] Illumina also notes that a nucleoside could be incorporated via chemical synthesis. The parties disagree as to whether the '537 patent covers chemical synthesis as opposed to just enzymatic synthesis. It is unnecessary to resolve this claim construction issue at this stage.

(ii) Full Scope of Azido Group.

Qiagen argues that Claim 1 (and dependent Claims 2–5 and 8) of the '537 patent are invalid because they encompass a broad set of modified nucleotides that have a "protecting group compris[ing] an azido group," while an "azido group" could refer to any of more than one thousand chemical groups. Moreover, because each base (A, C, T, or G) may have variant properties that affect their incorporation into a nucleic acid, different azido groups might work better in combination with different bases, compounding the scope of the claim (*id.* ¶¶ 98–100).

Although the mere possibility that a claim includes inoperative combinations is not a sufficient basis to invalidate a patent on its own, "if the number of inoperative combinations becomes significant, and in effect forces one of ordinary skill in the art to experiment unduly in order to practice the claimed invention, the claims might indeed be invalid." *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1576–77 (Fed. Cir. 1984). "General and vague" statements that certain claimed combinations might not work are insufficient to support a finding of lack of enablement. *See Union Carbide Chems. & Plastics Tech. Corp. v. Shell Oil Co*, 308 F.3d 1167, 1186 (Fed. Cir. 2002).

True, the number of combinations encompassed within the claims is significant, but to date, Qiagen has failed to identify *even one* inoperative combination. Rather, it rests solely on the possibility that a large proportion of the total number of combinations *could* be inoperative. Thus, Qiagen is unlikely to show, by clear and convincing evidence, that Illumina failed to enable the full scope of the azido group.[4]

(iii) 2' Protecting Group.

Qiagen's final enablement argument concerns the fact that the '537 patent covers "protecting group attached via the 2' or 3' oxygen atom," but the specification offers only a general statement that a protecting group of sufficient size and charge to protect the 3' position would also work at the 2' position. Again, Qiagen has not identified a single embodiment that

[4] This particular argument would not affect Claim 6 (asserted herein), which provides the additional limitation of a particular azido group, azidomethyl. Claim 6 would not survive Qiagen's other two enablement arguments or its obviousness argument, but even if Qiagen succeeded on this argument, it would still need to succeed on a second invalidity argument to avoid liability.

would not work, nor has it provided any evidence beyond the conclusory statements of its experts that a skilled artisan would have needed to undertake undue experimentation to practice this aspect of the claimed invention. Illumina is also likely to overcome this argument.

* * *

This is a rare and powerful case for the unusual remedy of a preliminary injunction. The validity of the patent-in-suit has been affirmed by the Federal Circuit (albeit without considering all the add-on arguments asserted here), and Qiagen has ignored Illumina's infringement case. Although Qiagen's invalidity arguments are not frivolous, this order finds that Illumina is likely to defeat them, particularly in light of Qiagen's burden to prove invalidity with clear and convincing evidence. Thus, this order finds Illumina is likely to succeed on the merits and now turns to the equitable considerations for a preliminary injunction.

2. IRREPARABLE HARM.

Illumina argues that an injunction is warranted because Qiagen's introduction of the GeneReader could interfere with Illumina's brand reputation, usurp long-term business opportunities, and damage customer goodwill. Specifically, Illumina argues that Qiagen's GeneReader competes with Illumina's affordable desktop DNA sequencing products in the market for clinical laboratories (Van Oene Decl. ¶¶ 13–18).

The market for DNA sequencing in clinical laboratories is expected to grow substantially in the near future, and Qiagen has a foothold in that market due to its other product lines. Now, as the doors to the market have swung open, Qiagen seeks to usurp Illumina's position in that market with pirated technology. Moreover, potential customers cannot easily be recovered, inasmuch as laboratories purchase new DNA-sequencing equipment infrequently and irregularly, in part because laboratories must win government approval for use of any new DNA-sequencing equipment (*id.* ¶¶ 26–29, 45).

Finally, Qiagen seeks to offer an alternative pricing plan — different from the prevailing trend — whereby customers could rent a GeneReader and pay per use, rather than purchasing the device outright.

At this crucial inflection point in the development of the market for DNA sequencing equipment for clinical laboratories, Illumina would suffer irreparable harm if Qiagen were allowed to capture and define the market with pirated technology alongside its preexisting relationships and disruptive business model. *See Celsis In Vitro, Inc. v. CellzDirect, Inc.*, 664 F.3d 922, 931 (Fed. Cir. 2012). That harm could be compounded if Qiagen's products perform poorly — a serious prospect supported by the evidence herein (*see* Arnold Dep. at 112–13; Liaw Exh. JJ at 1).

Qiagen responds that Illumina's delay in seeking this injunction (more than four years after it first learned of Qiagen's GeneReader) undermines its contention that it would suffer irreparable harm absent an injunction. Not so. Qiagen's launch of the GeneReader was plagued by a series of false starts, delays, and reformulations. Moreover, the validity of the '537 patent hung in limbo until the Federal Circuit upheld the PTAB's decision on Intelligent Bio-Systems' IPR challenge — just weeks before Illumina commenced this action.

Illumina's decision to wait until Qiagen's launch of the product became imminent ensured its infringement case would not rest on shifting sands. Illumina's motion is well-timed, seeking to halt Qiagen's assault on the market at its inception, before it can irreparably change the face of the market.

After arguing that Illumina waited too long to seek this motion, Qiagen again reverses field and argues that the motion should be denied because Illumina has not *yet* suffered irreparable harm, pointing to internal Illumina documents suggesting that Qiagen would take a year or two to pose a threat (Liaw Decl., Exhs. GG at *16, JJ at 1). On the contrary, Illumina's internal documents suggest it *already* views Qiagen's products as a threat and expects Qiagen to become a more significant threat over the next two years absent an injunction (*id.*, Exh. JJ; Walter Reply Decl., Exh. 6). Moreover, Qiagen's own assessment of its prospects project it will become a significant player in the coming year (Arnold Tr. at 93–95).

The purpose of an injunction is not to remedy irreparable harm that has already occurred (plainly, it could not), but to prevent that harm from occurring in the first place. This is not a case in which Illumina has made mere conclusory statements that it will suffer irreparable harm,

but rather one in which Illumina has demonstrated a real risk that Qiagen could capture and redefine the market with its pirated technology. Compensation for lost sales will not adequately remedy the harm Qiagen could do to Illumina's business absent an injunction.

3. BALANCE OF HARDSHIPS .

In evaluating Illumina's request for a preliminary injunction, we must weigh the harm that it will suffer absent the injunction against the harm that Qiagen will incur if the injunction is granted. *Hybritech Inc. v. Abbot Labs.*, 849 F.2d 1446, 1457 (Fed. Cir. 1988).

Both parties are major corporations with multiple streams of revenue. Ironically, after arguing that Illumina would suffer no irreparable harm absent an injunction, Qiagen contends there would be "no way to know the opportunities" Qiagen itself lost during the injunction, and that it would be unable to recoup its investment in the development and marketing of the GeneReader (Defs.' Opp. at 25). But that is the price of its election "to build a business on a product found to infringe . . . ." *Windsurfing Int'l, Inc. v. AMF, Inc.*, 782 F.2d 995, 1003 n.12 (Fed. Cir. 1986). The balance of hardships weighs in favor of an injunction.

4. PUBLIC INTEREST.

"[A]bsent any other relevant concerns . . . the public is best served by enforcing patents that are likely valid and infringed." *Abbott Labs. v. Andrx Pharm., Inc.*, 452 F.3d 1331, 1348 (Fed. Cir. 2006). Qiagen argues that because its product is currently marketed for analysis of cancer-related genes, an injunction could negatively impact cancer research, but Illumina is poised to meet any demand for Qiagen's product in the wake of an injunction, so that argument is unavailing. Moreover, as clinical laboratories adopt Qiagen's product, they may grow to rely on the potentially-infringing technology, only to face their own liability following the likely result in this action. Thus, the public interest weighs in favor of an injunction.

CONCLUSION

For the reasons stated above, Illumina's motion for a preliminary injunction is **GRANTED** as follows:

> Defendants and their officers, agents, affiliates, employees, and attorneys, and all those persons acting or attempting to act in concert or participation with them, are enjoined from making, using, offering to sell, or selling within the United States, or

> importing into the United States, or marketing, promoting, or distributing the Qiagen GeneReader NGS System, as depicted in Plaintiffs' moving papers, or any related product that embodies the claims of U.S. Patent No. 7,566,537 ("the '537 Patent"), such as the GeneRead Sequencing Q Kit (1) or the GeneRead Sequencing Q Kit (4).
>
> Within **TWENTY ONE CALENDAR DAYS**, defendants must promptly notify all United States-based users of the Qiagen GeneReader NGS System, and/or any related product that embodies the claims of the '537 Patent, such as the GeneRead Sequencing Q Kit (1) or the GeneRead Sequencing Q Kit (4), about this order such that these users are duly noticed and bound by this order under Federal Rule of Civil Procedure 65(d)(2).

Qiagen asks that Illumina be required to submit a bond as security for the harm it will allegedly suffer by not being able to market the GeneReader during the pendency of this case pursuant to Rule 65(c). Within **FOURTEEN CALENDAR DAYS**, Illumina must post a bond of twenty million dollars as security for the injunction.

In light of the deluge of unauthorized supplemental materials filed by both sides in relation to this motion, this order hereby establishes a précis system for this case, as follows. Except for discovery disputes, no further motions may be filed in this action without prior written approval. A party seeking approval to file a motion must file a précis that summarizes the essence of the motion and explains its urgency. Any party opposing approval to file based on the précis may file an opposition by noon on the second business day following the day on which the précis was filed. Both the précis and the opposition must not exceed three pages, double-spaced, and may not contain footnotes or attachments. After considering the précis and the opposition (if any), the Court will either grant or deny leave to file the motion. If leave is granted, a briefing schedule and hearing date will be set.

IT IS SO ORDERED.

Dated: September 9, 2016.

WILLIAM ALSUP
UNITED STATES DISTRICT JUDGE

IN THE UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF CALIFORNIA

ILLUMINA, INC, and ILLUMINA CAMBRIDGE LTD.,

Plaintiffs,

v.

QIAGEN N.V., QIAGEN GmbH, QIAGEN GAITHERSBURG, INC., QIAGEN SCIENCES, LLC, QIAGEN INC. (USA), QIAGEN REDWOOD CITY, INC., AND INTELLIGENT BIO-SYSTEMS, INC.,

Defendants.
_____/

No. C 16-02788 WHA

ORDER GRANTING IN PART AND DENYING IN PART MOTION TO STAY PRELIMINARY INJUNCTION

An order granted Illumina's motion for a preliminary injunction (Dkt. No. 120). Qiagen moves to stay the preliminary injunction pending its expedited appeal of the order. Alternatively, defendants seek to stay the order in part as to (i) manufacturing the Gene Reader devices in the United States for sales outside the country and (ii) sales of nucleotides to existing customers using the Gene Reader device.

In seeking a stay of the injunction as a whole, Qiagen argues that the order granting the injunction inadequately addressed certain points raised in opposition to Illumina's motion. Although Qiagen's arguments largely revisit arguments already rejected in the order granting Illumina's motion for the preliminary injunction, two points are worth addressing here.

First, Qiagen argues that the order granting the preliminary injunction ignored the fact that the Federal Circuit found that it was of "no moment" that the prior art reference to azido

groups challenged therein taught that they would be removed with low efficiency (while the sequencing method taught by Ju and Tsien required high efficiency) because the claimed invention did not require removal. Critically, however, the Federal Circuit found that fact fatal to the claimed motivation to combine those references in the first place. *Intelligent Bio-Sys., Inc. v. Illumina Cambridge Ltd.*, 821 F.3d 1359, 1367 (Fed. Cir. 2016).

Here, Qiagen pointed to the declaration of its expert, Dr. Michael Metzker, in which he asserted that a skilled artisan *would have* been motivated to consult the chapters of Greene & Wuts concerning lower-efficiency protecting groups (including azido groups) when adapting Ju or Tsien for the purpose of labeling. Metzker asserted that "[w]hile efficient removal for the 3'-OH protecting group would always be desirable, a method of labeling would not require the high yield or quantitative (*i.e.* high efficiency) removal discussed in Ju and Tsien in the context of using the labeled nucleotide for sequencing" (Metzker Decl. ¶ 71). Nevertheless, he failed to state that at the time of the '537 patent a skilled artisan would have appreciated that fact, ignored that limitation in Ju or Tsien, and therefore looked beyond the aliphatic alcohols chapter in Green & Wuts to the chapter on phenols. Accordingly, it remains unlikely that Qiagen will succeed in clearly and convincingly showing that the '537 patent is obvious in light of Ju or Tsien and Greene & Wuts.

Second, Qiagen continues to assert its inconsistent position that this injunction will cause it irreparable harm while Illumina will suffer no irreparable harm absent the injunction. Although lost sales are possibly quantifiable, the order rested on the fact that Qiagen sought to redefine the market with disruptive pricing models at a crucial inflection point in the market. That risk remains a sufficient basis for denying Qiagen's request to stay the preliminary injunction as a whole.

On the other hand, Qiagen's request to stay the injunction as to its *current* customers does not pose as sharp a risk. Illumina's lost sales to those customers are easily quantifiable, and any irreparable harm in shifting those customers' expectations about pricing models has already been done. Moreover, the public interest and equity in allowing Qiagen's current

customers to continue to provide gene sequencing services without purchasing an entirely new system from Illumina favor staying the injunction as to those customers pending Qiagen's appeal.

Separately, Qiagen seeks clarification that the preliminary injunction does not preclude it from manufacturing the Gene Reader for the purpose of sales abroad. Qiagen argues that because the '537 patent claims a method, simply manufacturing the Gene Reader in the United States would not infringe the patent. Illumina agrees, provided that Qiagen does not perform quality control testing in the United States. (Qiagen avers it does not.) Illumina opposes Qiagen's request to clarify the scope of the objection solely because Qiagen did not object to the scope of Illumina's proposed order (which the order granting the preliminary injunction largely adopted).

Neither side briefed the proper scope of the injunction, and the Court will not hold Qiagen responsible for that failure. Thus, this order clarifies that the injunction does *not* extend to the manufacture of Qiagen's Gene Reader products for sale abroad, provided the quality control testing does not occur in the United States.

Accordingly, Qiagen's motion to stay the preliminary injunction in full is **DENIED**, but its motion is **GRANTED** as to supplying nucleotides to its existing customers — including customers that had already begun Qiagen's so-called "try and buy" program — and as to manufacturing in the United States for sales abroad.

IT IS SO ORDERED.

Dated: September 22, 2016.

WILLIAM ALSUP
UNITED STATES DISTRICT JUDGE

US007566537B2

(12) **United States Patent**
Balasubramanian et al.

(10) **Patent No.: US 7,566,537 B2**
(45) **Date of Patent: Jul. 28, 2009**

(54) **LABELLED NUCLEOTIDES**

(75) Inventors: **Shankar Balasubramanian**, Cambridge (GB); **Colin Barnes**, Nr. Saffron Walden (GB); **Xiaohai Liu**, Nr. Saffron Walden (GB); **John Milton**, Nr. Saffron Walden (GB); **Harold Swerdlow**, Nr. Saffron Walden (GB); **Xiaolin Wu**, Nr. Saffron Walden (GB)

(73) Assignee: **Illumina Cambridge Limited**, Nr. Saffron Walden (GB)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 152 days.

(21) Appl. No.: **11/301,578**

(22) Filed: **Dec. 13, 2005**

(65) **Prior Publication Data**

US 2006/0188901 A1 Aug. 24, 2006

Related U.S. Application Data

(62) Division of application No. 10/227,131, filed on Aug. 23, 2002, now Pat. No. 7,057,026.

(30) **Foreign Application Priority Data**

Dec. 4, 2001 (GB) 0129012.1

(51) **Int. Cl.**

C12Q 1/68	(2006.01)
C12P 19/34	(2006.01)
C12M 1/34	(2006.01)
C07H 21/00	(2006.01)

(52) **U.S. Cl.** **435/6**; 435/91.1; 435/287.2; 536/23.1

(58) **Field of Classification Search** 536/23.1; 435/6, 91.1, 287.2

See application file for complete search history.

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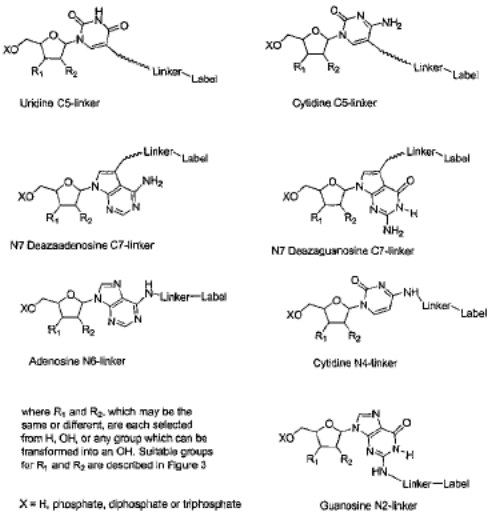
(74) *Attorney, Agent, or Firm*—Klauber & Jackson LLC

(57) **ABSTRACT**

Nucleosides and nucleotides are disclosed that are linked to detectable labels via a cleavable linker group.

15 Claims, 6 Drawing Sheets



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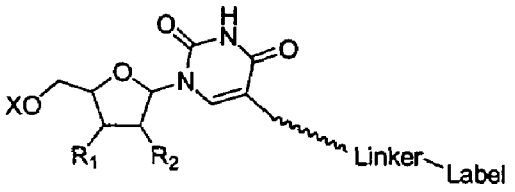
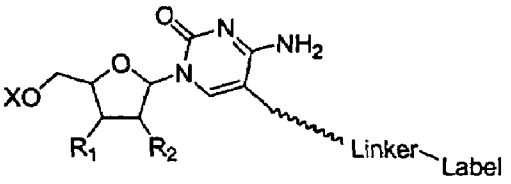


Uridine C5-linker



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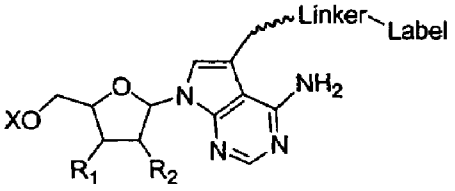
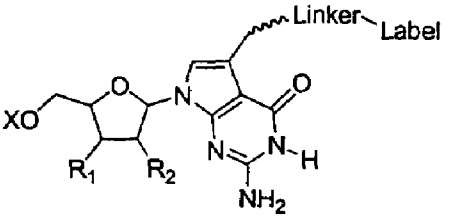
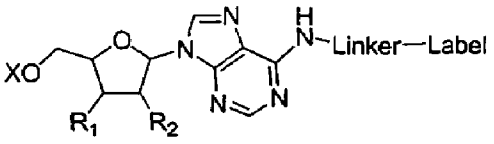


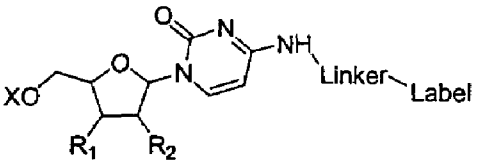
N7 Deazaadenosine C7-linker



N7 Deazaguanosine C7-linker

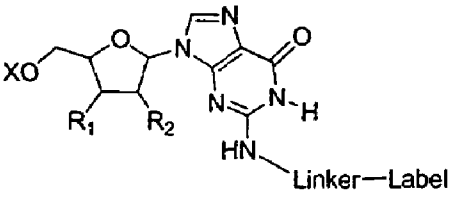


Adenosine N6-linker



Cytidine N4-linker

where R_1 and R_2, which may be the same or different, are each selected from H, OH, or any group which can be transformed into an OH. Suitable groups for R_1 and R_2 are described in Figure 3



Guanosine N2-linker

X = H, phosphate, diphosphate or triphosphate

Fig. 1

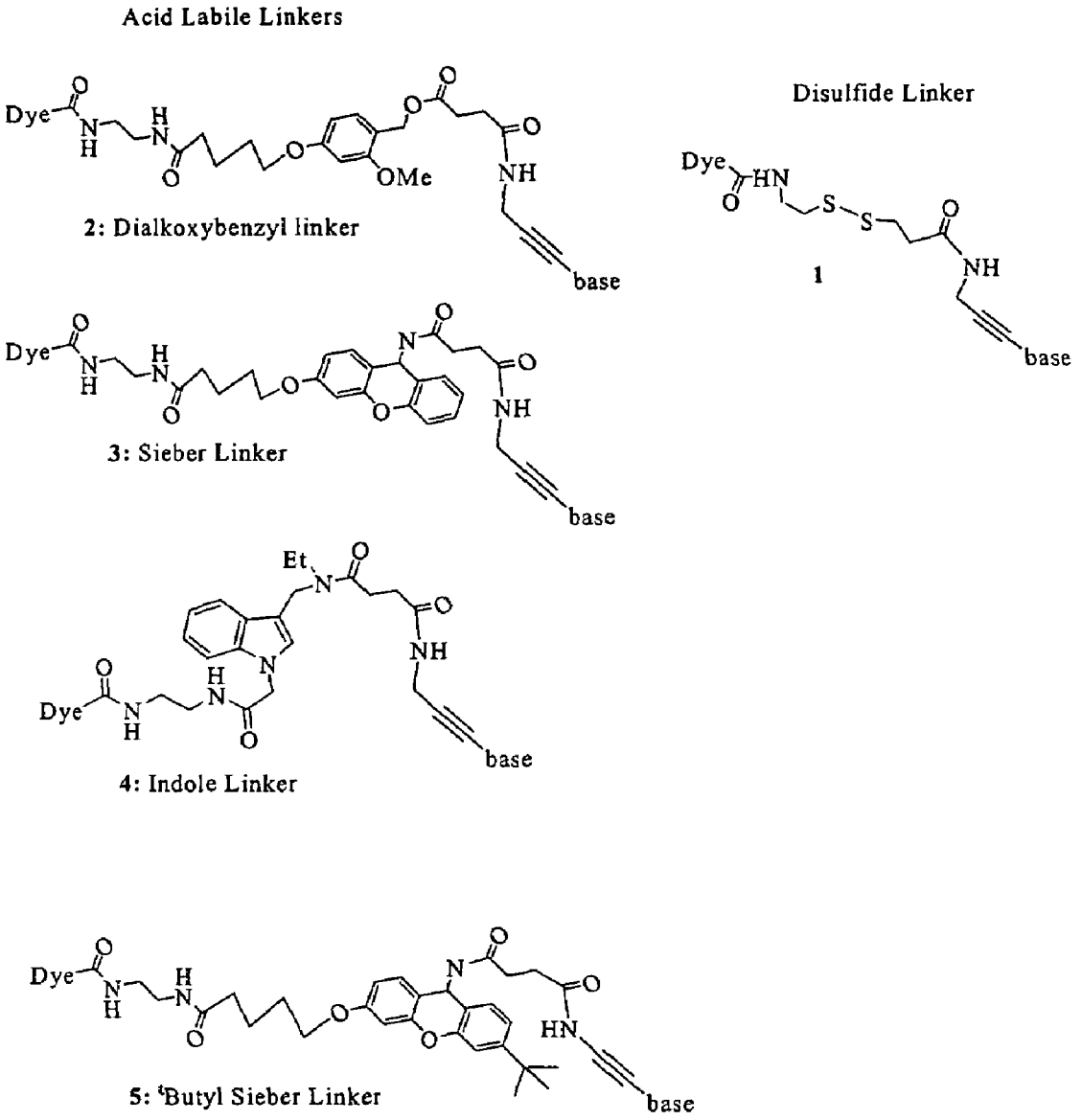
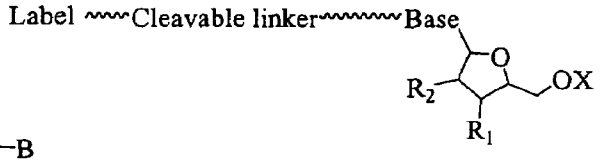
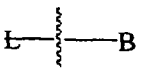
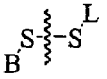

Acid Labile Linkers
Dye
OMe
base
2: Dialkoxybenzyl linker
3: Sieber Linker
Et
4: Indole Linker
5: tButyl Sieber Linker
Disulfide Linker
1

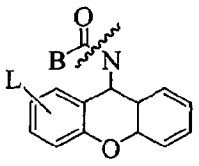
Fig. 2

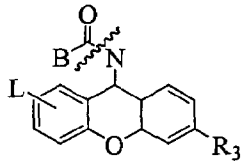


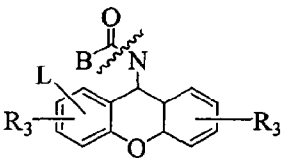


Cleavable linkers may include:







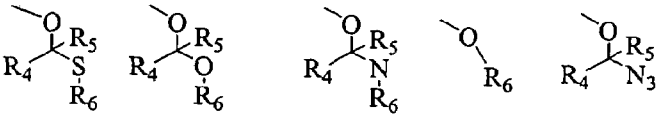


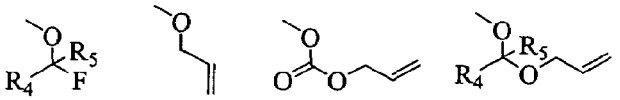
R3 represents one or more substituents independently selected from alkyl, alkoxy, amino or halogen

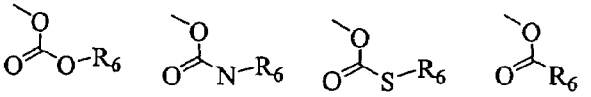
Alternatively, cleavable linkers may be constructed from any labile functionality used on the 3'-block

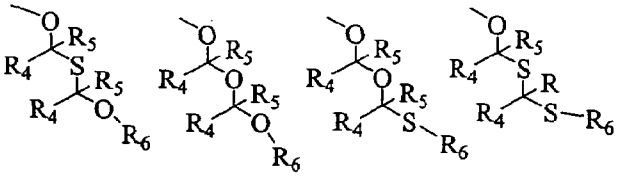
where R_1 and R_2, which may be the same or different, are each selected from H, OH, or any group which can be transformed into an OH, including a carbonyl

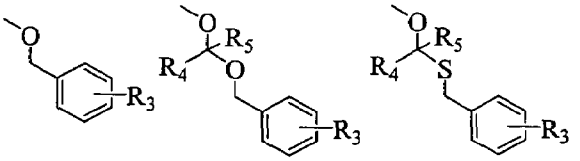
R_1 and R_2 groups may include







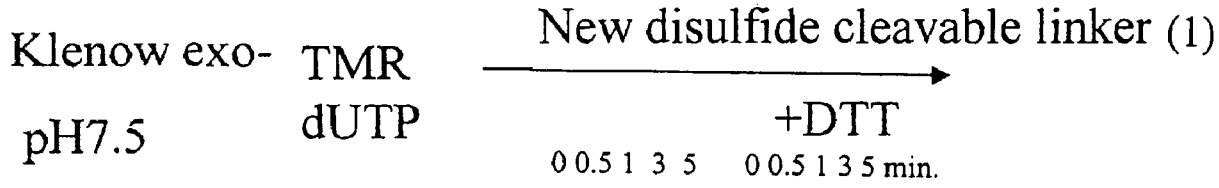




where R_4 is H or alkyl, R_5 is H or alkyl and R_6 is alkyl, cycloalkyl, alkenyl, cycloalkenyl or benzyl

and X is H, phosphate, diphosphate or triphosphate

Fig. 3

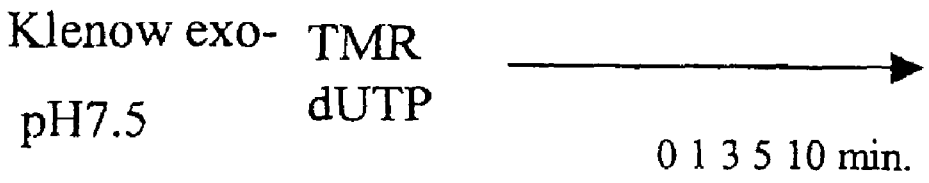


50mM Tris-HCl pH7.5, 10mM NaCl, 0.1mM EDTA
5mM MgCl2, 2uM dNTP-fluor, 100nM SHP 5T hairpin AG oligo,
Klenow exo- (Amersham-Joyce) 10units.

t = 0, 30s, 1,3, 5

Fig. 4

New Acid cleavable Sieber linker (3)

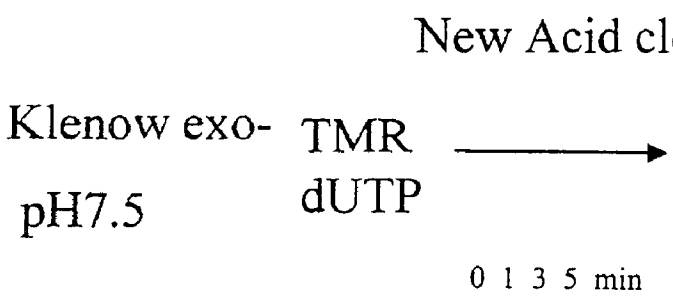


AG

50mM Tris-HCl pH7.5, 10mM NaCl, 2mM DTT, 0.1mM EDTA
5mM MgCl2, 2uM dNTP-fluor, 100nM SHP 5T hairpin AG oligo,
Klenow exo- (Amersham-Joyce) 10units.

t = 0, 1, 3, 5, 10

Fig. 5



50mM Tris-HCl pH7.5, 10mM NaCl, 2mM DTT, 0.1mM EDTA
5mM MgCl2, 2uM dNTP-fluor, 100nM SHP 5T hairpin AG oligo,
Klenow exo- (Amersham-Joyce) 10units.

t = 0, 1,3, 5

Fig. 6

LABELLED NUCLEOTIDES

RELATED APPLICATIONS

This application is a Divisional Application of U.S. application Ser. No. 10/227,131, now U.S. Pat. No. 7,057,026 filed Aug. 23, 2002 which in turn claims benefit of United Kingdom Application No. GB0129012.1, filed Dec. 4, 2001. The entire teachings of the above applications are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to labelled nucleotides. In particular, this invention discloses nucleotides having a removable label and their use in polynucleotide sequencing methods.

BACKGROUND

Advances in the study of molecules have been led, in part, by improvement in technologies used to characterise the molecules or their biological reactions. In particular, the study of the nucleic acids DNA and RNA has benefited from developing technologies used for sequence analysis and the study of hybridisation events.

An example of the technologies that have improved the study of nucleic acids, is the development of fabricated arrays of immobilised nucleic acids. These arrays consist typically of a high-density matrix of polynucleotides immobilised onto a solid support material. See, e.g., Fodor et al., *Trends Biotech.* 12:19-26, 1994, which describes ways of assembling the nucleic acids using a chemically sensitized glass surface protected by a mask, but exposed at defined areas to allow attachment of suitably modified nucleotide phosphoramidites. Fabricated arrays can also be manufactured by the technique of "spotting" known polynucleotides onto a solid support at predetermined positions (e.g., Stimpson et al., *Proc. Natl. Acad. Sci. USA* 92:6379-6383, 1995).

A further development in array technology is the attachment of the polynucleotides to the solid support material to form single molecule arrays. Arrays of this type are disclosed in International Patent App. WO 00/06770. The advantage of these arrays is that reactions can be monitored at the single molecule level and information on large numbers of single molecules can be collated from a single reaction.

For DNA arrays to be useful, the sequences of the molecules must be determined. U.S. Pat. No. 5,302,509 discloses a method to sequence polynucleotides immobilised on a solid support. The method relies on the incorporation of 3'-blocked bases A, G, C and T having a different fluorescent label to the immobilised polynucleotide, in the presence of DNA polymerase. The polymerase incorporates a base complementary to the target polynucleotide, but is prevented from further addition by the 3'-blocking group. The label of the incorporated base can then be determined and the blocking group removed by chemical cleavage to allow further polymerisation to occur.

Welch et al. (*Chem. Eur. J.* 5(3):951-960, 1999) describes the synthesis of nucleotide triphosphates modified with a 3'-O-blocking group that is photolabile and fluorescent. The modified nucleotides are intended for use in DNA sequencing experiments. However, these nucleotides proved to be difficult to incorporate onto an existing polynucleotide, due to an inability to fit into the polymerase enzyme active site.

Zhu et al. (*Cytometry* 28:206-211, 1997) also discloses the use of fluorescent labels attached to a nucleotide via the base group. The labelled nucleotides are intended for use in fluorescence in situ hybridisation (FISH) experiments, where a series of incorporated labelled nucleotides is required to produce a fluorescent "bar code".

SUMMARY OF THE INVENTION

In the present invention, a nucleoside or nucleotide molecule is linked to a detectable label via a cleavable linker group attached to the base, rendering the molecule useful in techniques using Labelled nucleosides or nucleotides, e.g., sequencing reactions, polynucleotide synthesis, nucleic acid amplification, nucleic acid hybridization assays, single nucleotide polymorphism studies, and other techniques using enzymes such as polymerases, reverse transcriptases, terminal transferases, or other DNA modifying enzymes. The invention is especially useful in techniques that use Labelled dNTPs, such as nick translation, random primer labeling, end-labeling (e.g., with terminal deoxynucleotidyltransferase), reverse transcription, or nucleic acid amplification. The molecules of the present invention are in contrast to the prior art, where the label is attached to the ribose or deoxyribose sugar, or where the label is attached via a non-cleavable linker.

According to a first aspect of the invention, a nucleotide or nucleoside molecule, or an analog thereof, has a base that is linked to a detectable label via a cleavable linker.

The invention features a nucleotide or nucleoside molecule, having a base that is linked to a detectable label via a cleavable linker. The base can be a purine, or a pyrimidine. The base can be a deazapurine. The molecule can have a ribose or deoxyribose sugar moiety. The ribose or deoxyribose sugar can include a protecting group attached via the 2' or 3' oxygen atom. The protecting group can be removed to expose a 3'-OH. The molecule can be a deoxyribonucleotide triphosphate. The detectable label can be a fluorophore. The linker can be an acid labile linker, a photolabile linker, or can contain a disulphide linkage.

The invention also features a method of labeling a nucleic acid molecule, where the method includes incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule, where the nucleotide or nucleoside molecule has a base that is linked to a detectable label via a cleavable linker. The incorporating step can be accomplished via a terminal transferase, a polymerase or a reverse transcriptase. The base can be a purine, or a pyrimidine. The base can be a deazapurine. The nucleotide or nucleoside molecule can have a ribose or deoxyribose sugar moiety. The ribose or deoxyribose sugar can include a protecting group attached via the 2' or 3' oxygen atom. The protecting group can be removed to expose a 3'-OH group. The molecule can be a deoxyribonucleotide triphosphate. The detectable label can be a fluorophore. The linker can be an acid labile linker, a photolabile linker, or can contain a disulphide linkage. The detectable label and/or the cleavable linker can be of a size sufficient to prevent the incorporation of a second nucleotide or nucleoside into the nucleic acid molecule.

In another aspect, the invention features a method for determining the sequence of a target single-stranded polynucleotide, where the method includes monitoring the sequential incorporation of complementary nucleotides, where the nucleotides each have a base that is linked to a detectable label via a cleavable linker, and where the identity of each nucleotide incorporated is determined by detection of the label linked to the base, and subsequent removal of the label.

The invention also features a method for determining the sequence of a target single-stranded polynucleotide, where the method includes: (a) providing nucleotides, where the nucleotides have a base that is linked to a detectable label via a cleavable linker, and where the detectable label linked to each type of nucleotide can be distinguished upon detection from the detectable label used for other types of nucleotides; (b) incorporating a nucleotide into the complement of the target single stranded polynucleotide; (c)detecting the label of the nucleotide of (b), thereby determining the type of nucleotide incorporated; (d) removing the label of the nucle-

otide of (b); and (e) optionally repeating steps (b)-(d) one or more times; thereby determining the sequence of a target single-stranded polynucleotide.

In the methods described herein, each of the nucleotides can be brought into contact with the target sequentially, with removal of non-incorporated nucleotides prior to addition of the next nucleotide, where detection and removal of the label is carried out either after addition of each nucleotide, or after addition of all four nucleotides.

In the methods, all of the nucleotides can be brought into contact with the target simultaneously, i.e., a composition comprising all of the different nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label(s).

The methods can comprise a first step and a second step, where in the first step, a first composition comprising two of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where in the second step, a second composition comprising the two nucleotides not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where the first steps and the second step can be optionally repeated one or more times.

The methods described herein can also comprise a first step and a second step, where in the first step, a composition comprising one of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where in the second step, a second composition. comprising the three nucleotides not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where the first steps and the second step can be optionally repeated one or more times.

The methods described herein can also comprise a first step and a second step, where in the first step, a first composition comprising three of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where in the second step, a composition comprising the nucleotide not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and where the first steps and the second step can be optionally repeated one or more times.

In a further aspect, the invention features a kit, where the kit includes: (a) individual the nucleotides, where each nucleotide has a base that is linked to a detectable label via a cleavable linker, and where the detectable label linked to each nucleotide can be distinguished upon detection from the detectable label used for other three nucleotides; and (b) packaging materials therefor. The kit can further include an enzyme and buffers appropriate for the action of the enzyme.

The nucleotides/nucleosides are suitable for use in many different DNA-based methodologies, including DNA synthesis and DNA sequencing protocols.

According to another aspect of the invention, a method for determining the sequence of a target polynucleotide comprises monitoring the sequential incorporation of complementary nucleotides, wherein the nucleotides comprise a detectable label linked to the base portion of the nucleotide via a cleavable linker, incorporation is detected by monitoring the label, and the label is removed to permit further nucleotide incorporation to occur.

DESCRIPTION OF THE DRAWINGS

FIG. **1** shows examplary nucleotide structures useful in the invention. For each structure, X can be H, phosphate, diphosphate or triphosphate. R_1 and R_2 can be the same or different, and can be selected from H, OH, or any group which can be transformed into an OH, including, but not limited to, a carbonyl. Some suitable functional groups for R_1 and R_2 include the structures shown in FIG. **3**.

FIG. **2** shows structures of linkers useful in the invention, including (1) disulfide linkers and acid labile linkers, (2) dialkoxybenzyl linkers, (3) Sieber linkers, (4) indole linkers and (5) t-butyl Sieber linkers.

FIG. **3** shows some functional molecules useful in the invention, including some cleavable linkers and some suitable hydroxyl protecting groups. In these structures, R_1 and R_2 may be the same of different, and can be H, OH, or any group which can be transformed into an OH group, including a carbonyl. R_3 represents one or more substituents independently selected from alkyl, alkoxyl, amino or halogen groups. Alternatively, cleavable linkers may be constructed from any labile functionality used on the 3'-block. R_4 and R_5 can be H or alkyl, and R_6 can be alkyl, cycloalkyl, alkenyl, cycloalkenyl or benzyl. X can be H, phosphate, diphosphate or triphosphate.

FIG. **4** shows a denaturing gel showing the incorporation of the triphosphate of Example 1 using Klenow polymerase.

FIG. **5** shows a denaturing gel showing the incorporation of the triphosphate of Example 3 using Klenow polymerase.

FIG. **6** shows a denaturing gel showing the incorporation of the triphosphate of Example 4 using Klenow polymerase.

DETAILED DESCRIPTION

The present invention relates to nucleotides and nucleosides that are modified by attachment of a label via a cleavable linker, thereby rendering the molecule useful in techniques where the labelled molecule is to interact with an enzyme, such as sequencing reactions, polynucleotide synthesis, nucleic acid amplification, nucleic acid hybridization assays, single nucleotide polymorphism studies, techniques using enzymes such as polymerase, reverse transcriptase, terminal transferase, techniques that use Labelled dNTPs (e.g., nick translation, random primer labeling, end-labeling (e.g., with terminal deoxynucleotidyltransferase), reverse transcription, or nucleic acid amplification).

As is known in the art, a "nucleotide" consists of a nitrogenous base, a sugar, and one or more phosphate groups. In RNA, the sugar is a ribose, and in DNA is a deoxyribose, i. e., a sugar lacking a hydroxyl group that is present in ribose. The nitrogenous base is a derivative of purine or pyrimidine. The purines are adenosine (A) and guanidine (G), and the pyrimidines are cytidine (C) and thymidine (T) (or in the context of RNA, uracil (U)). The C-1 atom of deoxyribose is bonded to N-1 of a pyrimidine or N-9 of a purine. A nucleotide is also a phosphate ester of a nucleoside, with esterification occurring on the hydroxyl group attached to C-5 of the sugar. Nucleotides are usually mono, di- or triphosphates.

A "nucleoside" is structurally similar to a nucleotide, but are missing the phosphate moieties. An example of a nucleoside analog would be one in which the label is linked to the base and there is no phosphate group attached to the sugar molecule.

Although the base is usually referred to as a purine or pyrimidine, the skilled person will appreciate that derivatives and analogs are available which do not alter the capability of the nucleotide or nucleoside to undergo Watson-Crick base

pairing. "Derivative" or "analog" means a compound or molecule whose core structure is the same as, or closely resembles that of, a parent compound, but which has a chemical or physical modification, such as a different or additional side groups, which allows the derivative nucleotide or nucleoside to be linked to another molecule. For example, the base can be a deazapurine. The derivatives should be capable of undergoing Watson-Crick pairing. "Deivative" and "analog" also mean a synthetic nucleotide or nucleoside derivative having modified base moieties and/or modified sugar moieties. Such derivatives and analogs are discussed in, e.g., Scheit, *Nucleotide Analogs* (John Wiley & Son, 1980) and Uhlman et al., *Chemical Reviews* 90:543-584, 1990. Nucleotide analogs can also comprise modified phosphodiester linkages, including phosphorothioate, phosphorodithioate, alkylphosphonate, phosphoranilidate and phosphoramidate linkages. The analogs should be capable of undergoing Watson-Crick base pairing. "Derivative" and "analog", as used herein, may be used interchangeably, and are encompassed by the terms "nucleotide" and "nucleoside" as defined herein.

The present invention can make use of conventional detectable labels. Detection can be carried out by any suitable method, including fluorescence spectroscopy or by other optical means. The preferred label is a fluorophore, which, after absorption of energy, emits radiation at a defined wavelength. Many suitable fluorescent labels are known. For example, Welch et al. (*Chem. Eur. J.* 5(3):951-960, 1999) discloses dansyl-functionalised fluorescent moieties that can be used in the present invention. Zhu et al. (*Cytometry* 28:206-211, 1997) describes the use of the fluorescent labels Cy3 and Cy5, which can also be used in the present invention. Labels suitable for use are also disclosed in Prober et al. (*Science* 238:336-341, 1987); Connell et al. (*BioTechniques* 5(4):342-384, 1987), Ansorge et al. (*Nucl. Acids Res.* 15(11): 4593-4602, 1987) and Smith et al. (*Nature* 321:674, 1986). Other commercially available fluorescent labels include, but are not limited to, fluorescein, rhodamine (including TMR, texas red and Rox), alexa, bodipy, acridine, coumarin, pyrene, benzanthracene and the cyanins.

Multiple labels can also be used in the invention. For example, bi-fluorophore FRET cassettes (*Tet. Letts.* 46:8867-8871, 2000) are well known in the art and can be utilised in the present invention. Multi-fluor dendrimeric systems (*J. Amer. Chem. Soc.* 123:8101-8108, 2001) can also be used.

Although fluorescent labels are preferred, other forms of detectable labels will be apparent as useful to those of ordinary skill. For example, microparticles, including quantum dots (Empodocles, et al., *Nature* 399:126-130, 1999), gold nanoparticles (Reichert et al., *Anal. Chem.* 72:6025-6029, 2000), microbeads (Lacoste et al., *Proc. Natl. Acad. Sci USA* 97(17):9461-9466, 2000), and tags detectable by mass spectrometry can all be used.

Multi-component labels can also be used in the invention. A multi-component label is one which is dependent on the interaction with a further compound for detection. The most common multi-component label used in biology is the biotin-streptavidin system. Biotin is used as the label attached to the nucleotide base. Streptavidin is then added separately to enable detection to occur. Other multi-component systems are available. For example, dinitrophenol has a commercially available fluorescent antibody that can be used for detection.

The label (or label and linker construct) can be of a size or structure sufficient to act as a block to the incorporation of a further nucleotide onto the nucleotide of the invention. This permits controlled polymerization to be carried out. The block can be due to steric hindrance, or can be due to a combination of size, charge and structure.

The invention will be further described with reference to nucleotides. However, unless indicated otherwise, the reference to nucleotides is also intended to be applicable to nucleosides. The invention will also be further described with reference to DNA, although the description will also be applicable to RNA, PNA, and other nucleic acids, unless otherwise indicated.

The modified nucleotides of the invention use a cleavable linker to attach the label to the nucleotide. The use of a cleavable linker ensures that the label can, if required, be removed after detection, avoiding any interfering signal with any labelled nucleotide incorporated subsequently.

Cleavable linkers are known in the art, and conventional chemistry can be applied to attach a linker to a nucleotide base and a label. The linker can be cleaved by any suitable method, including exposure to acids, bases, nucleophiles, electrophiles, radicals, metals, reducing or oxidising agents, light, temperature, enzymes etc. Suitable linkers can be adapted from standard chemical blocking groups, as disclosed in Greene & Wuts, *Protective Groups in Organic Synthesis*, John Wiley & Sons. Further suitable cleavable linkers used in solid-phase synthesis are disclosed in Guillier et al. (*Chem. Rev.* 100:2092-2157, 2000).

The use of the term "cleavable linker" is not meant to imply that the whole linker is required to be removed from the nucleotide base. The cleavage site can be located at a position on the linker that ensures that part of the linker remains attached to the nucleotide base after cleavage.

The linker can be attached at any position on the nucleotide base provided that Watson-Crick base pairing can still be carried out. In the context of purine bases, it is preferred if the linker is attached via the 7 position of the purine or the preferred deazapurine analogue, via an 8-modified purine, via an N-6 modified adenosine or an N-2 modified guanine. For pyrimidines, attachment is preferably via the 5 position on cytidine, thymidine or uracil and the N-4 position on cytosine. Suitable nucleotide structures are shown in FIG. **1**. For each structure in FIG. **1**, X can be H, phosphate, diphosphate or triphosphate. R_1 and R_2 can be the same or different, and can be selected from H, OH, or any group which can be transformed into an OH, including, but not limited to, a carbonyl. Some suitable functional groups for R_1 and R_2 include the structures shown in FIG. **3**.

Suitable linkers are shown in FIG. **2** and include, but are not limited to, disulfide linkers (1), acid labile linkers (2, 3, 4 and 5; including dialkoxybenzyl linkers (e.g., 2), Sieber linkers (e.g., 3), indole linkers (e.g., 4), t-butyl Sieber linkers (e.g., 5)), electrophilically cleavable linkers, nucleophilically cleavable linkers, photocleavable linkers, cleavage under reductive conditions, oxidative conditions, cleavage via use of safety-catch linkers, and cleavage by elimination mechanisms.

A. Electrophilically Cleaved Linkers.

Electrophilically cleaved linkers are typically cleaved by protons and include cleavages sensitive to acids. Suitable linkers include the modified benzylic systems such as trityl, p-alkoxybenzyl esters and p-alkoxybenzyl amides. Other suitable linkers include tert-butyloxycarbonyl (Boc) groups and the acetal system (e.g., as is shown in FIG. **3** as O—C(R_4)(R_5)—O—R_6.

The use of thiophilic metals, such as nickel, silver or mercury, in the cleavage of thioacetal or other sulphur-containing protecting groups can also be considered for the preparation of suitable linker molecules.

B. Nucleophilically Cleaved Linkers.

Nucleophilic cleavage is also a well recognised method in the preparation of linker molecules. Groups such as esters that are labile in water (i.e., can be cleaved simply at basic pH) and groups that are labile to non-aqueous nucleophiles, can be used. Fluoride ions can be used to cleave silicon-oxygen bonds in groups such as triisopropyl silane (TIPS) or t-butyldimethyl silane (TBDMS).

C. Photocleavable Linkers.

Photocleavable linkers have been used widely in carbohydrate chemistry. It is preferable that the light required to activate cleavage does not affect the other components of the modified nucleotides. For example, if a fluorophore is used as the label, it is preferable if this absorbs light of a different wavelength to that required to cleave the linker molecule. Suitable linkers include those based on O-nitrobenyl compounds and nitroveratryl compounds. Linkers based on benzoin chemistry can also be used (Lee et al., *J. Org. Chem.* 64:3454-3460, 1999).

D. Cleavage Under Reductive Conditions

There are many linkers known that are susceptible to reductive cleavage. Catalytic hydrogenation using palladium-based catalysts has been used to cleave benzyl and benzyloxycarbonyl groups. Disulphide bond reduction is also known in the art.

E. Cleavage Under Oxidative Conditions

Oxidation-based approaches are well known in the art. These include oxidation of p-alkoxybenzyl groups and the oxidation of sulphur and selenium linkers. The use of aqueous iodine to cleave disulphides and other sulphur or selenium-based linkers is also within the scope of the invention.

F. Safety-Catch Linkers

Safety-catch linkers are those that cleave in two steps. In a preferred system the first step is the generation of a reactive nucleophilic center followed by a second step involving an intra-molecular cyclization that results in cleavage. For example, levulinic ester linkages can be treated with hydrazine or photochemistry to release an active amine, which can then be cyclised to cleave an ester elsewhere in the molecule (Burgess et al., *J. Org. Chem.* 62:5165-5168, 1997).

G. Cleavage by Elimination Mechanisms

Elimination reactions can also be used. For example, the base-catalysed elimination of groups such as Fmoc and cyanoethyl, and palladium-catalysed reductive elimination of allylic systems, can be used.

As well as the cleavage site, the linker can comprise a spacer unit. The spacer distances the nucleotide base from the cleavage site or label. The length of the linker is unimportant provided that the label is held a sufficient distance from the nucleotide so as not to interfere with any interaction between the nucleotide and an enzyme.

The modified nucleotides can also comprise additional groups or modifications to the sugar group. For example, a dideoxyribose derivative, lacking two oxygens on the ribose ring structure (at the 2' and 3' positions), can be prepared and used as a block to further nucleotide incorporation on a growing oligonucleotide strand. The ribose ring can also be modified to include a protecting group at the 3' position or a group that can be transformed or modified to form a 3' OH group. The protecting group is intended to prevent nucleotide incorporation onto a nascent polynucleotide strand, and can be removed under defined conditions to allow polymerisation to occur. In contrast to the prior art, there is no detectable label attached at the ribose 3' position. This ensures that steric hindrance with the polymerase enzyme is reduced, while still allowing control of incorporation using the protecting group.

The skilled person will appreciate how to attach a suitable protecting group to the ribose ring to block interactions with the 3'-OH. The protecting group can be attached directly at the 3' position, or can be attached at the 2' position (the protecting group being of sufficient size or charge to block interactions at the 3' position). Alternatively, the protecting group can be attached at both the 3' and 2' positions, and can be cleaved to expose the 3' OH group.

Suitable protecting groups will be apparent to the skilled person, and can be formed from any suitable protecting group disclosed in Green and Wuts, supra. Some examples of such protecting groups are shown in FIG. **3**. The protecting group should be removable (or modifiable) to produce a 3' OH group. The process used to obtain the 3' OH group can be any suitable chemical or enzymic reaction.

The labile linker may consist of functionality cleavable under identical conditions to the block. This will make the deprotection process more efficient as only a single treatment will be required to cleave both the label and the block. Thus the linker may contain functional groups as described in FIG. **3**, which could be cleaved with the hydroxyl functionality on either the residual nucleoside or the removed label. The linker may also consist of entirely different chemical functionality that happens to be labile to the conditions used to cleave the block.

The term "alkyl" covers both straight chain and branched chain alkyl groups. Unless the context indicates otherwise, the term "alkyl" refers to groups having 1 to 8 carbon atoms, and typically from 1 to 6 carbon atoms, for example from 1 to 4 carbon atoms. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-pentyl, 2-pentyl, 3-pentyl, 2-methyl butyl, 3-methyl butyl, and n-hexyl and its isomers.

Examples of cycloalkyl groups are those having from 3 to 10 ring atoms, particular examples including those derived from cyclopropane, cyclobutane, cyclopentane, cyclohexane and cycloheptane, bicycloheptane and decalin.

Examples of alkenyl groups include, but are not limited to, ethenyl (vinyl), 1-propenyl, 2-propenyl (allyl), isopropenyl, butenyl, buta-1,4-dienyl, pentenyl, and hexenyl.

Examples of cycloalkenyl groups include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl and cyclohexenyl.

The term alkoxy refers to C_{1-6} alkoxy unless otherwise indicated: —OR, wherein R is a C_{1-6}alkyl group. Examples of C_{1-6} alkoxy groups include, but are not limited to, —OMe (methoxy), —OEt (ethoxy), —O(nPr) (n-propoxy), —O(iPr) (isopropoxy), —O(nBu) (n-butoxy), —O(sBu) (sec-butoxy), —O(iBu) (isobutoxy), and —O(tBu) (tert-butoxy).

The term amino refers to groups of type NR^1R^2, wherein R^1 and R^2 are independently selected from hydrogen, a C_{1-6} alkyl group (also referred to as C_{1-6} alkylamino or di-C_{1-6} alkylamino).

The term "halogen" as used herein includes fluorine, chlorine, bromine and iodine.

The nucleotide molecules of the present invention are suitable for use in many different methods where the detection of nucleotides is required.

DNA sequencing methods, such as those outlined in U.S. Pat. No. 5,302,509 can be carried out using the nucleotides.

A method for determining the sequence of a target polynucleotide can be carried out by contacting the target polynucleotide separately with the different nucleotides to form the complement to that of the target polynucleotide, and detecting the incorporation of the nucleotides. Such a method makes use of polymerisation, whereby a polymerase enzyme extends the complementary strand by incorporating the correct nucleotide complementary to that on the target. The polymerisation reaction also requires a specific primer to initiate polymerisation.

For each cycle, the incorporation of the labelled nucleotide is carried out by the polymerase enzyme, and the incorporation event is then determined. Many different polymerase enzymes exist, and it will be evident to the person of ordinary skill which is most appropriate to use. Preferred enzymes include DNA polymerase I, the Klenow fragment, DNA polymerase III, T4 or T7 DNA polymerase, Taq polymerase or vent polymerase. A polymerase engineered to have specific properties can also be used.

The sequencing methods are preferably carried out with the target polynucleotide arrayed on a solid support. Multiple target polynucleotides can be immobilised on the solid support through linker molecules, or can be attached to particles, e.g., microspheres, which can also be attached to a solid support material.

The polynucleotides can be attached to the solid support by a number of means, including the use of biotin-avidin interactions. Methods for immobilizing polynucleotides on a solid support are well known in the art, and include lithographic techniques and "spotting" individual polynucleotides in defined positions on a solid support. Suitable solid supports are known in the art, and include glass slides and beads, ceramic and silicon surfaces and plastic materials. The support is usually a flat surface although microscopic beads (microspheres) can also be used and can in turn be attached to another solid support by known means. The microspheres can be of any suitable size, typically in the range of from 10 nm to 100 nm in diameter. In a preferred embodiment, the polynucleotides are attached directly onto a planar surface, preferably a planar glass surface. Attachment will preferably be by means of a covalent linkage. Preferably, the arrays that are used are single molecule arrays that comprise polynucleotides in distinct optically resolvable areas, e.g., as disclosed in International App. No. WO 00/06770.

The sequencing method can be carried out on both single polynucleotide molecule and multi-polynucleotide molecule arrays, i.e., arrays of distinct individual polynucleotide molecules and arrays of distinct regions comprising multiple copies of one individual polynucleotide molecule. Single molecule arrays allow each individual polynucleotide to be resolved separately. The use of single molecule arrays is preferred. Sequencing single molecule arrays non-destructively allows a spatially addressable array to be formed.

The method makes use of the polymerisation reaction to generate the complementary sequence of the target. The conditions necessary for polymerisation to occur will be apparent to the skilled person.

To carry out the polymerase reaction it will usually be necessary to first anneal a primer sequence to the target polynucleotide, the primer sequence being recognised by the polymerase enzyme and acting as an initiation site for the subsequent extension of the complementary strand. The primer sequence may be added as a separate component with respect to the target polynucleotide. Alternatively, the primer and the target polynucleotide may each be part of one single stranded molecule, with the primer portion forming an intramolecular duplex with a part of the target, i.e., a hairpin loop structure. This structure may be immobilised to the solid support at any point on the molecule. Other conditions necessary for carrying out the polymerase reaction, including temperature, pH, buffer compositions etc., will be apparent to those skilled in the art.

The modified nucleotides of the invention are then brought into contact with the target polynucleotide, to allow polymerisation to occur. The nucleotides may be added sequentially, i.e., separate addition of each nucleotide type (A, T, G or C), or added together. If they are added together, it is preferable for each nucleotide type to be labelled with a different label.

This polymerisation step is allowed to proceed for a time sufficient to allow incorporation of a nucleotide.

Nucleotides that are not incorporated are then removed, for example, by subjecting the array to a washing step, and detection of the incorporated labels may then be carried out.

Detection may be by conventional means, for example if the label is a fluorescent moiety, detection of an incorporated base may be carried out by using a confocal scanning microscope to scan the surface of the array with a laser, to image a fluorophore bound directly to the incorporated base. Alternatively, a sensitive 2-D detector, such as a charge-coupled detector (CCD), can be used to visualise the individual signals generated. However, other techniques such as scanning near-field optical microscopy (SNOM) are available and may be used when imaging dense arrays. For example, using SNOM, individual polynucleotides may be distinguished when separated by a distance of less than 100 nm, e.g., 10 nm to 10 μm. For a description of scanning near-field optical microscopy, see Moyer et al., *Laser Focus World* 29:10, 1993. Suitable apparatus used for imaging polynucleotide arrays are known and the technical set-up will be apparent to the skilled person.

After detection, the label may be removed using suitable conditions that cleave the linker.

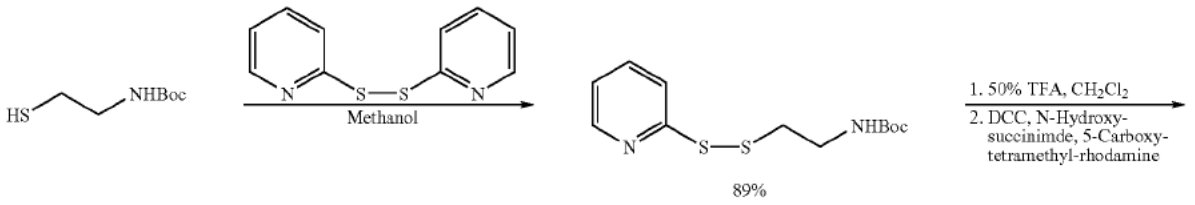
The use of the modified nucleotides is not limited to DNA sequencing techniques, and other techniques, including polynucleotide synthesis, DNA hybridisation assays and single nucleotide polymorphism studies, may also be carried out using nucleotides of the invention. Any technique that involves the interaction between a nucleotide and an enzyme may make use of the molecules of the invention. For example, the molecule may be used as a substrate for a reverse transcriptase or terminal transferase enzyme.

Suitable structures are described in the following Examples and are shown in the accompanying drawings.

EXAMPLES

Example 1

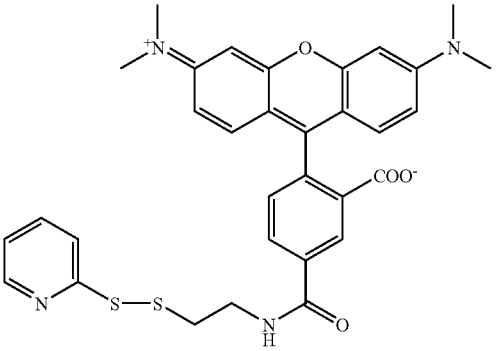
Synthesis of Disulfide Linker



89%

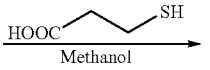
1a

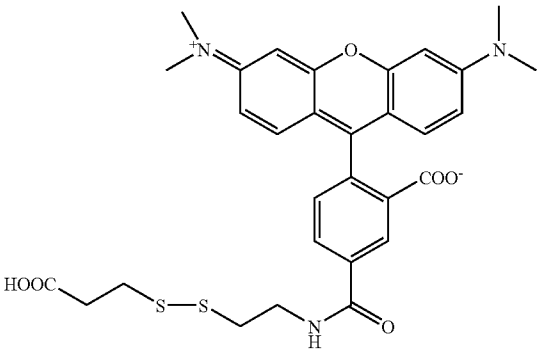
-continued



90%

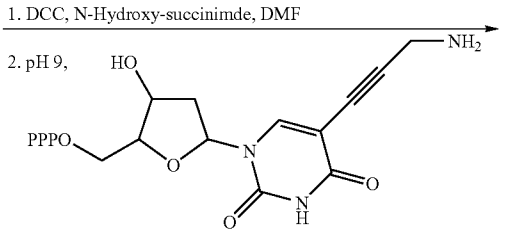
1b

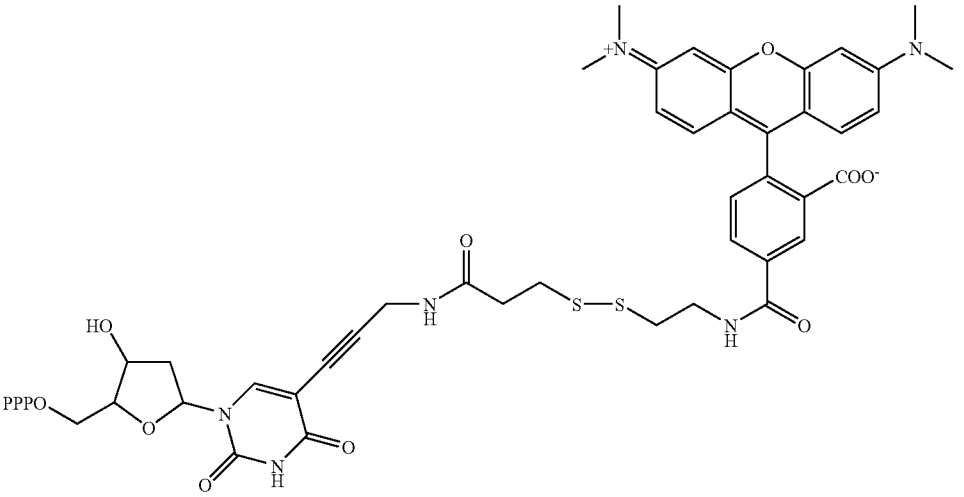




80%

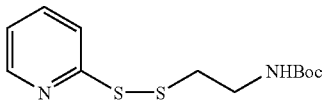
1c





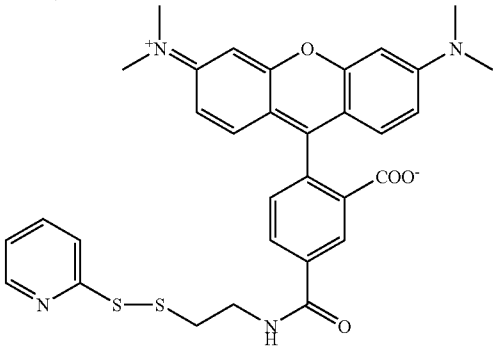
1

1a



tButyl-N-(2-mercaptoethyl) carbamate (3 mmol, 0.5 mL) was added dropwise to a solution of 1.32 g (6.0 mmol) aldrithiol in 15 mL MeOH. After 1.5 h the reaction had gone to completion and the solvent was evaporated. The crude product was purified by chromatography on silica with ethyl acetate:petroleum ether (1:4). Product 1a was obtained as a slightly yellow oil (0.76 g, 2.67 mmol, 89%). ^{1}H NMR (500 Mhz, D_6-DMSO): δ=1.38 (s, 9 H, tBu), 2.88 (t, J=6.6 Hz, 2 H, SCH_2) 3.20 (q, J=6.6 Hz, 2 H, $C\underline{H}_2NH$), 7.02 (bs, 1 H, NH), 7.24 (ddd, J=7.3 Hz, J=4.9 Hz, J=1.0Hz, 1 H, H-5), 7.77 (dt, J=8.1 Hz, J=1.0 Hz, 1 H, H-3), 7.82 (ddd, J=8.1 Hz, J=7.4 Hz, J=1.8 Hz, 1 H, H-4), 8.46 (ddd, J=4.9 Hz, J=1.8 Hz, J=1.0 Hz, 1 H, H-6).

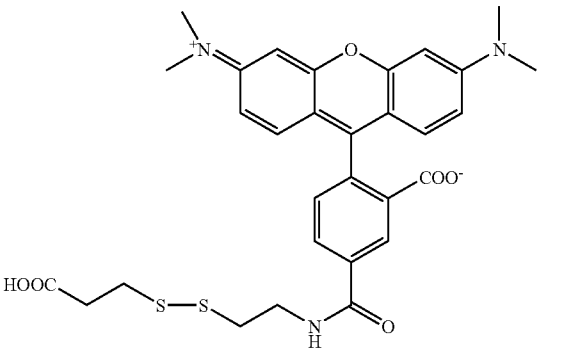
1b



To deprotect the amine of 1a, 17 mg of 1a (60 μmol) was dissolved in a mixture of 0.5 mL DCM and 0.5 mL trifluoracetic acid. This mixture was stirred for 2.5 h at rt and then the solvents were removed under reduced pressure. The residue was three times redissolved in 2 mL DCM and evaporated to dryness. The deprotected product was dried under high vacuum for 3 h and then dissolved in 1 mL dry DMF. It was assumed that the deprotection had gone to completion.

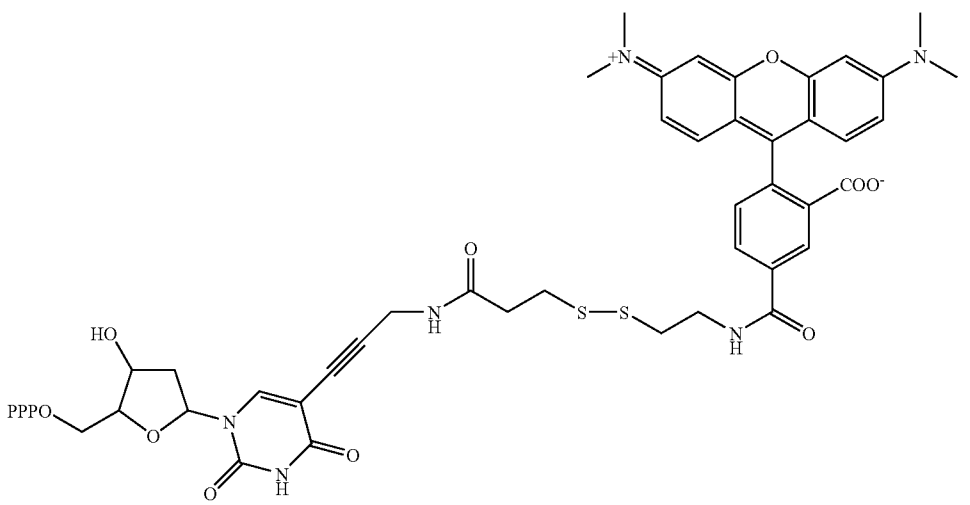
To a solution of 15 mg 5-carboxy tetra methyl rhodamine (35 μmol) in 2 mL DMF were added 8.0 mg N-hydroxy succinimide (70 μmol) and 7.8 mg DCC (38 μmol). The mixture was stirred for 6 h in the dark. Then 22 μl DIPEA (126 μmol) and the solution of deprotected 1a in 1 mL DMF were added. After stirring the reaction mixture overnight in the dark, the solvent was removed under reduced pressure. The residue was dissolved in DCM and washed with saturated NaCl solution. After drying over $MgSO_4$ the crude mixture was purified on silica with $CHCl_3$:MeOH (3:1) as solvent. 1b was isolated as a dark red solid in 90% yield (19.2 mg, 31.4 μmol). ^{1}H NMR (500 MHz, D_6-DMSO): δ=3.09 (t, J=6.7 Hz, 2 H, SCH_2), 3.63 (q, J=6.2 Hz, 2 H, $C\underline{H}_2NH$), 6.48-6.53 (m, 6 H, H-Anthracene), 7.23-7.26 [m, 1 H, H-5 (pyridine)], 7.32 (d, J=7.9 Hz, 1 Hz, H-3), 7.81-7.82 [m, 2 H, H-3+H-4 (pyridine)], 8.21 (d, J=7.9 Hz, 1 H, H-4), 8.43 (s, 1 H, H-6), 8.47 [dt, J=4.7 Hz, J=1.3 Hz, 1 H, H-6 (pyridine)], 9.03 (t, J=5.2 Hz, 1 H, NH).

1c



Mercaptopropionic acid (20.6 μmol, 1.8 μl) was added to a solution of 19.6 mg 1b (32.7 μmol) in 2 mL MeOH. The mixture was stirred for 2.5 h in the dark. The solvent was removed under reduced pressure. The crude product was purified by chromatography on silica with $CHCl_3$:MeOH:AcOH 15:1:0.5 as the solvent mixture. 15.5 mg (26 μmol, 80%) dark red crystals 1c could be isolated. ^{1}H NMR (500 MHz, D_2O): δ=2.53 (t, J=7.0 Hz, 2 H, $C\underline{H}_2COOH$), 2.88 (t, J=7.0 Hz, 2 H, $C\underline{H}_2CH_2COOH$), 2.96-2.99 (m, 2 H, $C\underline{H}_2CH_2NH$), 3.73 (t, J=6.3 Hz, 2 H, $C\underline{H}_2NH$), 6.53 (d, J=2.4 Hz, 2 H, H-Anthracene), 6.81 (dd, J=9.5 Hz, J=4.5 Hz, 2 H, H-Anthracene), 7.12 (d, J=9.5 Hz, 2 H, H-Anthracene), 7.48 (d, J=7.9 Hz, 1 H, H-3), 7.95 (dd, J=8.1 Hz, J=1.9 Hz, 1 H, H-2) 8.13 (d, J=1.9 Hz, 1 H, H-1). +ve electro spray ($C_{30}H_{31}N_3O_6S_2$): expected 593.17; found 594.3 [M+H], 616.2 [M+Na].

1



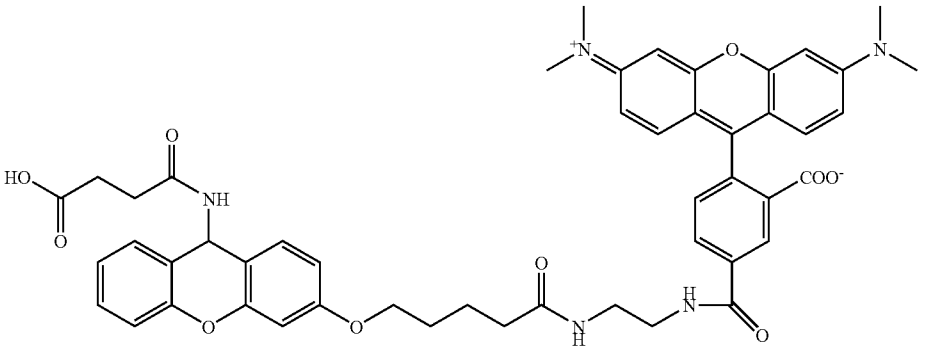
To a solution of 25.8 mg 1c (43.4 μmol) in 3 mL DMF (dry) were added 9.9 mg N-hydroxy succinimide (86.8 μmol) and 9.7 mg DCC (47.1 μmol). The mixture was stirred in the dark for 5 h at room temperature and then put in the fridge overnight. The mixture was filtered through a plug of cotton wool in a new flask and to this was added a solution of 865 μl propargylamino dUTP (14.7 μmol, 17 μmol in 1 mL H_2O) and 3 mL sodium borate buffer (0.1 M solution, pH 9). The mixture was stirred overnight. After removal of solvents the residue was dissolved in as little water as possible and purified by HPLC. A Zorbax C18 column was used with 0.1 M triethyl ammonium bicarbonate (TEAB) and acetonitrile as buffers. ^{31}P NMR (400 MHz, D_2O): δ=−4.73 (d), −9.93 (d), 19.03 (t). -ve electro spray ($C_{42}H_{47}N_6O_{19}P_3S_2$ assuming 4 H^+ counter ions): expected 1096.16; found 1092.9. UV in Water: $\lambda_{(max)}$=555 nm $A_{(555)}$=0.885 (c=0.036 μmol).

Triphosphate (1) was successfully incorporated using Klenow DNA polymerase. The reaction was performed in the following conditions: 50 mM Tris.HCl (pH 7.5), 10 mM NaCl, 2 mM DTT, 0.1 mM EDTA, 5 mM $MgCl_2$, 2 μM compound 3, 100 nM DNA template (previously labelled with P32 and T4 polynucleotide kinase) and 10 units of commercial exo-Klenow (Amersham Corp., Arlington Heights, Ill., USA). The DNA templates were self-complementary hairpins (5'-TACCgTCgACgTCgACgCTggCgAgCgTgCTgCggTTTTT(C6-amino)TTACCgCAgCACgCTCgCCAgCg; SEQ ID NO: 1). The reaction was performed in 100 μL volume at 37° C. with timepoints taken at 0, 1, 3, 5 and 10 min. The reaction products were electrophoresed down a denaturing (8 M urea) 20% polyacrylamide gel and imaged on a typhoon phosphorimager. Complete single base extension was seen in 1 minute indicating efficient polymerase incorporation (disulfide linker gel, FIG. **3**). A second set of lanes is shown in which the material is exposed to DTT after the incorporation. A different band shift can be seen which shows removal of the dye from the DNA construct, thus a cycle of polymerase incorporation and cleavage has been shown using this disulfide compound.

Example 2

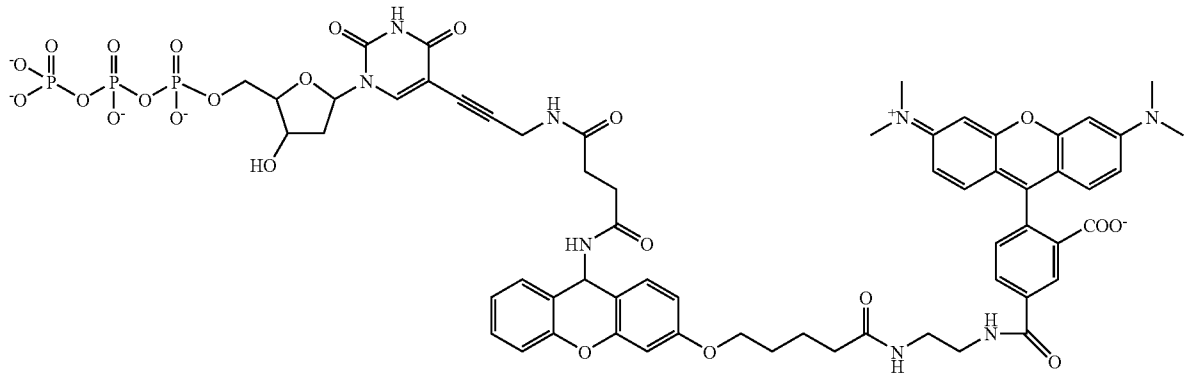
Synthesis of TMR-Sieber Linker Free Acid

5-[-9-[9-(fluorenyl-methyloxycarbonyl)amino]xanthen-3-yl]valeric acid, (42.8 mg, 80 μmol) was stirred at room temperature with disuccinimidyl carbonate (22.5 mg, 88 μmol) and N,N-dimethyl aminopyridine (10.8 mg, 88 μmol) in DMF. After 5 minutes, mono-5-carboxy TMR ethylene diamine (198.9 mg, 40 μmol) was added followed by DIPEA (13.9 μl, 80 μmol). The reaction was stirred at room temperature. After 2 hrs, the reaction mixture was diluted with dichloromethane (100 mL) and the resulting solution was extracted with 1 M aqueous potassium dihydrogen phosphate (50 mL). The DCM layer was separated and evaporated under reduced pressure. The residue was purified by a short column chromatography. The fractions eluting with 40% methanol in chloroform were collected and evaporated under reduced pressure. The residue was then dissolved in dry DMF (1 mL) and N-(2-mercaptoethyl)aminomethyl polystyrene (200 mg, 400 μmol) and DBU (12 μl, 80 μmol). After 10 minutes at room temperature, the resins were filtered off and rinsed with dry DMF (1 mL). All the filtrates were combined and then added to a solution of succinic anhydride (80 mg, 800 μmol), DIPEA (139 μl, 800 μmol) and DMAP (9.8 mg, 80 μmol) in DMF (1 mL). The reaction mixture was then stirred at room temperature. After overnight (16 hrs), all the solvents were evaporated under reduced pressure and the residue was purified by a short column chromatography. The title compound eluted with 30% methanol in chloroform was obtained as purple powders (22 mg, overall yield 63%). ^{1}HNMR [D_6-DMSO]: 8.82 (1H, t, J 5.4, ex.), 8.75 (1H, d, J 8.9, ex.), 8.42 (1H, d, J 1.5), 8.20 (1H, dd, J 8.0 and 1.5), 7.95 (1H, t, J 5.9, ex.), 7.34 (1H, d, J 7.3), 7.30-7.27 (2H, m), 7.21 (1H, d, J 8.5), 7.16-7.07 (2H, m), 6.68 (1H, dd, J 8.8 and 2.5), 6.65 (1H, d, J 2.4), 6.49-6.43 (6H, m), 6.18 (1H, d, J 5.6), 3.95 (1H, t, J 5.9), 3.39-3.36 (2H, m), 3.30-3.27 (2H, m), 2.92 (12H, s), 2.37-2.33 (2H, m), 2.14(2H, t, J 7.2) and 1.70-1.62 (4H, m). MS[(ES(+)], m/z 868.5 (MH^+).



Example 3

Synthesis of TMR-Sieber Linker-dUTP (3)

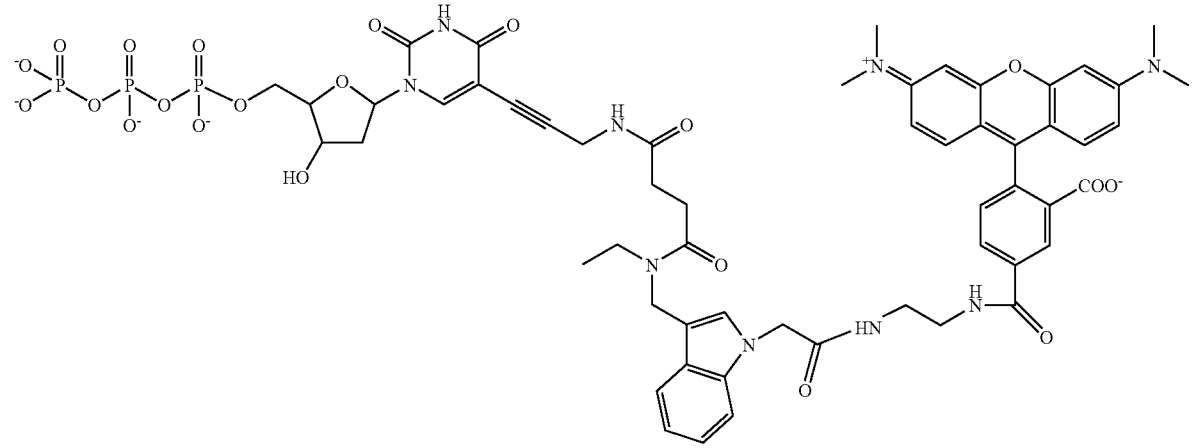


TMR-sieber linker free acid (4.34 mg, 5 μmol) was stirred with disuccinimidyl carbonate (1.74 mg, 7.5 μmol) and N,N-dimethyl aminopyridine (0.92 mg, 7.5 μmol) in DMF (1 mL) at room temperature. After 10 minutes, all the reaction mixture was added to tetra-(tri-butylammonium) salt of 5-(3-aminopropynyl)-2'-deoxyuridine-5'-triphosphate (10 μmol). The reaction was stirred at room temperature for 4 hrs and stored in the fridge overnight. The reaction mixture was then diluted with chilled water (10 mL) and all the resulting solution was applied onto a short column of DEAE A-25. The column was initially eluted with 0.1 M TEAB buffer and then 0.7 M TEAB buffer. The 0.7 M TEAB eluents were collected and evaporated under reduced pressure. The residue was co-evaporated with MeOH (2×10 mL) and then purified by preparative HPLC. The title compound was obtained as triethylammonium salt in 31% yield (based on the quantification of TMR at 555 nm in water (pH 7)). ^{1}HNMR in D_2O indicated two diastereoisomers, due to the sieber linker moiety and there were approximately three triethylammonium count ions. ^{1}HNMR [D_2O]: 8.18 (1H, m), 8.06 (1H, m), 7.76 (0.55H, s), 7.74 (0.45H, s), 7.36-7.09 (5H, m), 6.89-6.72 (3H, m), 6.59-6.37 (5H, m), 6.12 (0.55H, t, J 6.6), 6.05 (0.45H, t, J 6.6), 5.99 (0.45H, d, J 2.5), 5.91 (1.1H, m), 5.88 (0.45H, s), 4.49 (0.55H, m), 4.43 (0.45H, m), 4.00-3.35 (9H, m), 3.30-2.95 (32H, m), 2.65-2.52 (4H, m), 2.25-2.05 (4H, m), 1.62-1.42 (4H, m) and 1.23 (27H, t, J 7.3). ^{31}P [D_2O]: −9.91 ($^{\gamma}$P, d, J 19.2), [−11.08($^{\alpha}$P, d, J20.1) and −11.30 ($^{\alpha}$P, d, J 20.1), due to two diastereoisomers] and −22.57 ($^{\beta}$P, m). MS[(ES(−)], m/z 1369.1 (M^-).

Triphosphate (3) was successfully incorporated using Klenow DNA polymerase. The reaction was performed in the following conditions: 50 mM Tris.HCl (pH 7.5), 10 mM NaCl, 2 mM DTT, 0.1 mM EDTA, 5 mM $MgCl_2$, 2 μM compound 3, 100 nM DNA template (previously labelled with P32 and T4 polynucleotide kinase) and 10 units of commercial exo-Klenow (Amersham Corp. Arlington Heights, Illinois, USA). The DNA templates were self-complementary hairpins (5'-TACCgTCgACgTCgACgCTggCgAgCgT-gCTgCggTTTTT(C6-amino)TTACCgCAgCACgCTCgC-CAgCg; SEQ ID NO:1). The reaction was performed in 100 μL volume at 37° C. with timepoints taken at 0, 1, 3, 5 and 10 min. The reaction products were electrophoresed down a denaturing (8 M urea) 20% polyacrylamide gel and imaged on a typhoon phosphorimager. Complete single base extension was seen in 1 minute indicating efficient polymerase incorporation (Sieber linker gel, FIG. **4**).

Example 4

Synthesis of TMR-Indole Linker-dUTP (4)



Triphosphate (4) was successfully incorporated using Klenow DNA polymerase. The reaction was performed in the following conditions: 50 mM Tris.HCl (pH 7.5), 10 mM NaCl, 2 mM DTT, 0.1 mM EDTA, 5 mM $MgCl_2$, 2 μM compound 3, 100 nM DNA template (previously labelled with P32 and T4 polynucleotide kinase) and 10 units of commercial exo-Klenow (Amersham Corp., Arlington Heights, Ill., USA). The DNA templates were self-complementary hairpins (5'-TACCgTCgACgTCgACgCTggCgAgCgTgCTgCggTTTTT(C6-amino)TTACCgCAgCACgCTCgCCAgCg; SEQ ID NO:1). The reaction was performed in 100 μL volume at 37° C. with timepoints taken at 0, 1, 3, 5 and 10 min. The reaction products were electrophoresed down a denaturing (8 M urea) 20% polyacrylamide gel and imaged on a typhoon phosphorimager. Complete single base extension was seen in 1 minute indicating efficient polymerase incorporation (indole linker gel, FIG. **5**).

All patents, patent applications, and published references cited herein are hereby incorporated by reference in their entirety. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

SEQUENCE LISTING

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<222> LOCATION: 40
<223> OTHER INFORMATION: thymidine with an amino modification on the C6 position

<400> SEQUENCE: 1

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gcg 63

What is claimed is:

1. A method of labeling a nucleic acid molecule, the method comprising incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule, wherein the nucleotide or nucleoside molecule has a base that is linked to a detectable label via a cleavable linker and the nucleotide or nucleoside molecule has a ribose or deoxyribose sugar moiety, wherein the ribose or deoxyribose sugar moiety comprises a protecting group attached via the 2' or 3' oxygen atom, and said protecting group can be modified or removed to expose a 3' OH group and the protecting group comprises an azido group.

2. The method of claim **1**, wherein said incorporating is accomplished via a terminal transferase, a polymerase or a reverse transcriptase.

3. The method of claim **1**, wherein the base is a deazapurine.

4. The method of claim **1**, wherein the nucleotide is a deoxyribonucleotide triphosphate.

5. The method of claim **1**, wherein the label is a fluorophore.

6. The method according to claim **1**, wherein the protecting group is CH_2N_3.

7. A method of labeling a nucleic acid molecule, the method comprising incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule, wherein the nucleotide or nucleoside molecule has a base that is linked to a detectable label via a cleavable linker and the nucleotide or nucleoside molecule has a ribose or deoxyribose sugar moiety, wherein the ribose or deoxyribose sugar moiety comprises a protecting group attached via the 2' or 3' oxygen atom, and said protecting group can be modified or removed to expose a 3' OH group and the protecting group and cleavable linker are removable under identical conditions.

8. The method according to claim **1**, further comprising detecting the detectable label and cleaving the cleavable linker.

9. A method of labeling a nucleic acid molecule, the method comprising:

a) incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule, wherein the nucleotide or nucleoside molecule has a base that is linked to a detectable label via a cleavable linker, wherein the nucleotide or nucleoside molecule has a ribose or deoxyribose sugar moiety that comprises a protecting group attached via the 2' or 3' oxygen atom, and said protecting group can be modified or removed to expose a 3' OH group and the protecting group comprises an azido group;

b) detecting the detectable label and cleaving the cleavable linker, wherein the protected hydroxyl functionality and the protecting group are cleavable under identical conditions; and

c) repeating steps a) and b) at least once.

10. The method according to claim **7**, wherein the cleavable linker and the protecting group both comprise an allyl moiety.

11. The method of claim **7**, wherein said incorporating is accomplished via a terminal transferase, a polymerase or a reverse transcriptase.

12. The method of claim **7**, wherein the base is a deazapurine.

13. The method of claim **7**, wherein the nucleotide is a deoxyribonucleotide triphosphate.

14. The method of claim **7**, wherein the label is a fluorophore.

15. A method of labeling a nucleic acid molecule, the method comprising:

a) incorporating into the nucleic acid molecule a nucleotide or nucleoside molecule, wherein the nucleotide or nucleoside molecule has a base that is linked to a detectable label via a cleavable linker, wherein the nucleotide or nucleoside molecule has a ribose or deoxyribose sugar moiety that comprises a protecting group attached via the 2' or 3' oxygen atom, and said protecting group can be modified or removed to expose a 3' OH group and the protecting group comprises an allyl moiety;

b) detecting the detectable label and cleaving the cleavable linker, wherein the protected hydroxyl functionality and the protecting group are cleavable under identical conditions; and

c) repeating steps a) and b) at least once.

* * * * *

PROOF OF SERVICE

The undersigned certifies that on October 21, 2016, true copies of the Corrected Brief of Defendants-Appellants Non-Confidential Version were served via e-mail on the counsel of record.

Dated: October 21, 2016 By: /s/ *Robert R. Baron, Jr.*
Robert R. Baron, Jr.

CERTIFICATE OF COMPLIANCE—BRIEF CONTAINING MATERIAL SUBJECT TO A PROTECTIVE ORDER

This Brief complies with the limitations set forth in FED. CIR. R. 28(d) and contains fourteen words (including numbers) marked as confidential.

Dated: October 21, 2016 By: /s/ *Robert R. Baron, Jr.*
Robert R. Baron, Jr.

CERTIFICATE OF COMPLIANCE WITH TYPE-VOLUME LIMITATION, TYPEFACE REQUIREMENTS, AND TYPE STYLE REQUIREMENTS

This brief contains 13,838 words, excluding the parts of the brief exempted by Federal Rule of Appellate Procedure 32(a)(7)(B)(iii).

This brief complies with the typeface requirements of Federal Rule of Appellate Procedure 32(a)(5) of Federal Rule of Appellate Procedure 28.1(e) and the type style requirements of Federal Rule of Appellate Procedure 32(a)(6).

This brief has been prepared in a proportionally spaced typeface using Microsoft Word 2010 in Times New Roman 14-point font.

Dated: October 21, 2016

By: /s/ *Robert R. Baron, Jr.*
Robert R. Baron, Jr.